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John Cairns, Jr., Paul V. McCormick, Matthew E. Arnegard, and Eric P. Smith

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University Center for Environmental and Hazardous Materials Studies
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0415

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This report presents the results of field studies which were designed to investigate the variability in periphyton sensitivity to stress. Chlorine and sulfuric acid were experimentally delivered to periphyton communities using chemical-diffusing substrates. Significant variations in chlorine sensitivity among ecosystems were generally not explained by differences in water quality characteristics, but may be related to differences in the inherent tolerance of algal taxa to chlorine exposure. Periphyton community biomass and oxygen consumption did not respond to decreases in pH as low as pH=3; taxonomic responses to this manipulation are still being analyzed. The report also describes a study of stress induced tolerance of periphyton communities to novel copper stress using chemical diffusers.

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John Cairns, Jr.

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and

Eric P. Smith

University Center for Environmental
& Hazardous Materials Studies

Virginia Polytechnic Institute
and
State University

Blacksburg, VA 24061-0415

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ABSTRACT

1. During the second year of this project, three in situ dosing studies were conducted to assess variation in biological sensitivity to common chemical stressors among several minimally impacted streams in the Blue Ridge and Southern Appalachian regions of southwestern Virginia.

2. This report presents results from two of the in situ studies, for which data has been collected, summarizes ongoing data analysis efforts, and outlines proposed research plans for the third year of the contract.

3. The response and recovery of stream periphyton assemblages to chlorine exposure indicated that ecosystems may vary substantially in their sensitivity to a novel stressor. Determination of the relative sensitivity of different streams to chlorine exposure was dependent upon the biological response parameters considered. Variations in sensitivity to chlorine among ecosystems were generally not explained by differences in water quality characteristics, but may be related to differences in the inherent ability of different species to tolerate chlorine exposure.

4. Sulfuric acid-diffusing substrates enabled experimental acidification of periphyton mats, exposure levels were measured using a modified, surface pH electrode. Periphyton community biomass and oxygen consumption did not respond to short term acidification (below pH 4 in one stream and below pH 3 in two other streams). Further analysis of the taxonomic responses of

the communities to this manipulation are currently in progress.

5. Periphyton communities in five "pristine" and five historically impacted streams received a novel toxic pollutant (cupric chloride) in an investigation of the influence of past stress (e.g., via community adaptation) on community sensitivity to copper. Preliminary observations indicate that previous exposure to metal toxicants other than copper reduces the impact of subsequent exposure to copper.

6. Future experiments will investigate whether this phenomenon, if supported by the final analysis of the data collected during the copper manipulation, is generalizable to toxicants in dissimilar classes of stress.

INTRODUCTION

This report summarizes research conducted during the second year of the grant AFOSR-91-0379 entitled "Measuring Variation in Ecosystem Sensitivity to Stress." The continuing focus of this work has been to provide experimental evidence to corroborate or refute the hypothesis that outwardly similar ecosystems (e.g., low-order, high gradient streams) exhibit significant differences in their sensitivity to anthropogenic (i.e., "novel") stressors and that this variation cannot be explained based on physicochemical differences (e.g., hardness) alone. The possibility that some ecosystems are inherently more sensitive than others to human impacts has not be studied in a comprehensive manner, although the finding from any such study could have major implications for resource management and conservation strategies at all levels of government.

Current environmental regulations (e.g., EPA Ambient Water Quality Criteria) acknowledge differences in susceptibility to impact among ecosystems on the basis of specific water chemistry components (e.g., hardness) that have been documented to affect the toxicity of certain chemical agents. Restated, some ecosystems, because of their chemical environment, are inherently more resistant to certain forms of impact than are others. Physical and biological properties may also contribute to variation in the ability of ecosystems to resist and recover from disturbance (e.g., Webster et al. 1983). Previous work conducted under contract from the AFOSR (Cairns and Niederlehner 1991)

indicated the potential for ecosystems to adapt to specific chemical stressors and, following exposure to one chemical, to exhibit increased resistance to a novel chemical stressor. Therefore, it is also possible that ecosystems exhibit adaptive mechanisms to resist changes in structure and function in response to chronic stress.

All ecosystems on the planet have been exposed to human activity of one form or another. The relative importance of innate versus adaptive mechanisms of ecosystem resistance to human disturbance is not known. In attempting to extrapolate earlier laboratory findings to the real world, several questions must be answered including the following: 1) do minimally impacted ecosystems, i.e., those having little previous exposure to human disturbance, exhibit different innate capacities to resist and recover from an anthropogenic impact?; 2) do ecosystems with a history of exposure to human activity exhibit greater resistance and resilience in response to a novel impact compared with minimally impacted systems, i.e., is there a general adaptive mechanism? The focus of this project is to provide evidence with which to address these questions and to suggest the biological and chemical processes that determine the extent of resistance and resilience to human disturbance.

The University Center for Environmental & Hazardous Materials Studies (UCE&HMS) efforts to address the above questions have focused on microbial responses in general, and periphyton (attached algal) responses specifically, for several

reasons. Periphyton are a ubiquitous and conspicuous biological component of aquatic ecosystems. Most aquatic systems contain a taxonomically diverse assemblage of algal populations, each exhibiting a somewhat unique array of sensitivities to different chemical stressors. Many species exhibit widespread distributions, both regionally and globally, thus allowing for direct comparisons of population responses among ecosystems. Algae account for the bulk of the primary production in most aquatic habitats and, thus, serve as the principal autochthonous energy base for aquatic food webs. Algae play key roles in nutrient spiralling by sequestering dissolved inorganic nutrients (e.g., carbon, nitrogen, and phosphorus) that might otherwise be lost from the ecosystem and converting these elements into organic forms. Thus, changes in the periphyton assemblage in response to chemical stressors can have important implications for energy flow and biogeochemical cycling within the ecosystem.

In order to increase the comparability of sensitivity estimates among ecosystems, we limited our study to one ecosystem type, forested mountain streams in the eastern United States. Flowing water systems have historically been important receptors and conduits of human waste and, today, are subjected to a wider array of anthropogenic impacts than perhaps any other ecosystem type. Therefore, an understanding of how these ecosystems respond to and adapt to stress is especially relevant to ecotoxicology and environmental science. The mountainous region of southwestern Virginia serves as the watershed for several

major rivers in the eastern United States. Headwater streams were selected for study in this region because they are a dominant feature of the landscape. Although most streams in this region have some history of disturbance by humans (e.g., logging), many are considered relatively pristine (i.e., minimally impacted) at present. Other streams are impacted to different degrees by a variety of human activities, ranging from point source inputs of toxic chemicals and treated sewage to nonpoint source pollutants from agriculture and livestock. Five minimally impacted streams were selected for study during the first year of the project and research at these sites is ongoing. During the second year of the study, additional stream sites were selected for study that are exposed to different levels of human disturbance. The reasons for their selection and a description of their use in a recent investigation are provided later in this report (see Ongoing Research and Future Avenues of Inquiry).

Objectives

The principal objectives of this three year study are to: 1) determine the extent of baseline variability in sensitivity to chemical stressors among minimally-impacted ecosystems; and 2) determine whether increased exposure to human disturbance affects the sensitivity of an ecosystem to a novel stressor.

The specific objectives of this report are to: 1) summarize the focus of work conducted during the second year of the study; 2) present the findings of two of the field studies conducted during the past fiscal year to assess variability in

susceptibility to chemical stressors among reference (i.e., minimally-impacted) ecosystems; 3) describe ongoing field experiments and associated work to be completed during the third year of the project.

Research Synopsis

Whereas the first year of the contract emphasized laboratory and field studies designed to standardize the dosing and sampling technique, the second year of research has focused on field studies designed to test some of the hypotheses originally proposed. Controlled field dosing studies were performed to assess variability in periphyton community response to two common chemical pollutants, sulfuric acid and chlorine, among five reference streams. Structural and functional responses were measured in order to provide a robust test of the null hypothesis that minimally-impacted (i.e., reference) ecosystems in similar geographical regions exhibit similar sensitivities to anthropogenic stressors. Beyond providing a statistical test of the above hypothesis, the experimental data also provided quantitative estimates of uncertainty associated with standard predictions (e.g., effect concentrations) of the hazard that commonly used chemicals pose to natural ecosystems. These results will be compared with previous laboratory and field studies to synthesize information concerning chemical impacts on ecosystems.

At present, a large-scale field study is being concurrently conducted in five relatively undisturbed streams and five streams

with documented histories of stress. Interspersion of water chemistry characteristics (e.g., hard water vs. soft water) and geographic location among the reference and impacted streams has been considered in site selection, in addition to the proximity and accessibility of these new sites. An experimental stressor, cupric chloride, has been delivered to algal communities in these streams. The purpose of the present year of investigation is to assess the influence of stress history on periphyton sensitivity to novel stressors (e.g., via genetic or physiological adaptation or community level taxonomic shifts). Specifically, the null hypothesis of no differences in mean sensitivities to copper between the reference streams and the impacted streams will be tested. A synopsis of the scientific rationale and experimental design for this study is given below (pp. 52-55).

SECTION II: VARIATION IN ECOSYSTEM SENSITIVITY TO CHLORINE

Introduction and Objectives

Chlorine is widely used as a biocide to remove or control bacteria, algae, and other organisms in municipal and industrial effluents, industrial cooling water, swimming pools, and drinking water (USEPA 1990). Chlorine released into the environment in its active form (hypochlorite) is not persistent as the hypochlorite ion readily reacts with oxidizable compounds that are ubiquitous in freshwater. Reactions between free residual chlorine and naturally occurring compounds (e.g., ammonia, humic materials, and nitrogenous compounds) result in the formation of a number of chlorinated byproducts, some of which (e.g., chloroform) exhibit carcinogenic and mutagenic properties (Ward 1974, Marouka and Jamanaka 1980). The relative proportions of different chemical species that comprise this total residual chlorine, which includes both free and combined forms, is affected by several water quality parameters including temperature, pH, ammonia, and humic acid content (see Melzian and Jaworski 1991).

Because of the extreme toxicity of hypochlorite to aquatic life and the potential for formation of toxic byproducts, strict water quality criteria have been adopted for this chemical. The current chronic criterion for total residual chlorine in freshwaters based on a four day average exposure is 11 ug/L (USEPA 1985).

There are several possible reasons why ecosystems might vary

in their sensitivity to chlorine exposure. There is a broad range of sensitivities among freshwater fish and invertebrates to chlorine exposure (USEPA 1985). Sensitivity to chlorine is also affected by several water quality parameters. There is evidence that the persistence and, therefore, the impact of chlorine in the environment increases with decreasing water temperature (Nacci et al. 1990). The pH of water also affects the speciation and reactivity of chlorine with other chemical compounds and aquatic life (APHA et al. 1985). Chlorine reacts readily with ammonia; therefore, increased concentrations of ammonia in the environment result in enhanced rates of removal of free residual chlorine with a concomitant increase in the rate of formation of certain byproducts (e.g., chloramines) that have the potential for bioaccumulation in aquatic organisms (Melzian and Jaworski 1991). The net result of reactions between chlorine and ammonia may be a lessening of the toxic effects of either compound as evidenced by the findings of Cairns et al. (1990), which showed that mixtures of the two chemicals were less inhibitory to protozoan assemblages than expected on the basis of additive toxic effects of each chemical alone. Although the effect of biological and chemical characteristics on chlorine toxicity remain poorly understood (Melzian and Jaworski 1991), the above studies indicate that considerable variation in ecosystem sensitivity to chlorine exposure may exist.

The objective of this experiment was to test for differences in sensitivity to chlorine among five streams in southwestern

Virginia. The five streams selected for study were all deemed to be minimally-impacted by human activity as evidence by their location and surrounding land use and the biological characteristics of the streams. In particular, none of these streams had a history of exposure to chlorine or other industrial or municipal effluents. Thus, the data collected in this experiment provided an indication of the range of expected response of reference ecosystems to a novel chemical stressor.

Methods

Study locations. The study was conducted in southwestern Virginia in the eastern Appalachian and Blue Ridge regions. Sampling locations in five watersheds were selected within these two regions to serve as a minimally-impacted (i.e., reference) site for assessing ecosystem sensitivity to chemical impact. Selection criteria included: 1) an absence of point source discharges upstream; 2) minimal impact from nonpoint sources (e.g., agriculture and animal husbandry), as evidenced by the nature of surrounding land use patterns and the presence of an intact riparian zone; 3) macroinvertebrate community composition based on EPA-approved sampling protocols (Plafkin et al., 1989). Inevitably, considerations such as ease of access and landowner permission had to be factored into the selection process. Using the above selection procedure, three tributaries of the Roanoke River (Blue Ridge Province), Bradshaw Creek, Goose Creek, and Rock Creek, and two tributaries of the New River (Appalachian

Province), John's Creek and Kimberling Creek, were selected for experimentation.

Diffuser and Metabolism Chamber Design. The design of the chemical diffusing substrates used in this study are described in great detail in the first year report and will only be summarized here. Substrates were constructed by sealing 10 cm x 10 cm, unglazed terra-cotta tiles over square holes cut in polystyrene tissue culture flasks using silicone sealant. This chamber design allowed for the measurement of periphyton metabolism using the oxygen-change method.

Light and dark chambers for measuring periphyton metabolism were constructed from transparent plexiglass to form a 500 ml chamber with an open end (100 cm2) that fit snugly over the clay substrate of the diffusers by means of a silicone gasket and several heavy duty rubber bands. The exterior of some metabolism chambers was sprayed with black paint to allow for estimates of periphyton respiration while other chambers were left untreated to allow for estimates of net photosynthesis (i.e., photosynthesis uncorrected for concurrent respiration).

Miniature, submersible pumps (Edmund Scientific) maintained flow through the metabolism chambers during incubations. Baffles were located at either end of the chamber in order to increase uniformity of flow across the surface of the enclosed periphyton mat. A plastic syringe, placed in-line with the pump, allowed aliquots of water to be removed during the incubation period without introducing air into the chamber. A flow interrupter

facilitated proper mixing in this portion of the chamber.

Multiple metabolism chambers were operated simultaneously using direct current from a rechargeable Dura Power II marine battery (Delco Remy), which was modulated with a square wave generator and a variable transformer to yield alternating current to insulated pairs of alligator clamps, wired in parallel. The entire circuit was housed in a weather-proof wooden box for easy transport to the field.

Experimental Design. Several chemical diffusing substrates were placed in each stream at the beginning of experimentation. Two 1 m^2 wooden pallets were first weighted to the bottom of each stream using rocks from the stream as anchors. Pool locations (current velocities around 10-20 cm/sec) were selected in each stream that were comparable in terms of depth and incident solar radiation. Thirty-six diffusers were attached to the pallets in each stream by means of velcro strips secured with epoxy. Each diffuser was preassigned a specific chlorine doses and diffusers were pseudorandomly (i.e., visually) arranged on the pallets on the basis of these treatment assignments.

All diffusers were initially filled with filter-sterilized (0.2 um) water from the appropriate stream and incubated in the streams for 21 days to allow for periphyton growth on the clay surface. Following this initial colonization period, eight replicate diffusers were removed from each stream and sampled for the structural and functional parameters described below (see Sampling Methods).

Following the colonization period and baseline measurements, diffusers were refilled with one of the following molar concentrations of chlorine as hypochlorite dissolved in filter-sterilized stream water: 0, 0.005, 0.01, 0.05, 0.1, 0.5, or 1.0. Diffusers were carefully detached from the pallet, removed from the stream, and tilted to one side in order to replace filtered stream water with test concentrations of chlorine while attempting to minimize disruption of the attached periphyton assemblage. The periphyton assemblage in all streams was dominated by a mucilaginous, diatom-based biofilm that exhibited reasonably good adherence to the clay substratum, and, consequently, little observable loss of biomass from the substratum was observed during the exchange process. Furthermore, because all diffusers were removed and refilled, even if biological disruptions (e.g., loss of biomass from the substrate) did occur, they would be similar among treatments.

Following a 7 day dosing period, the periphyton assemblages on four substrates of each chlorine treatment were sampled as described below to determine impacts associated with chlorine exposure. Immediately following these measurements, chlorine solutions in all remaining diffusers were replaced with control solutions (i.e., filter-sterilized water obtained from the same stream) and reattached to the pallets using procedures described above. After 7 more days of incubation, two additional substrates that had been exposed to each chlorine dose were sampled using the following procedure.

Sampling Methods. The sampling protocol developed for field studies included methods for obtaining both structural and functional measurements. These methods were detailed in the 1st year annual report, submitted in October, 1992. Functional measurements were performed using light and dark metabolism chambers to estimate changes in the rates of net photosynthesis (light chambers) and respiration (dark chambers) in response to increased chemical dosing. Methods standardized for performing these measurements involved the determination of changes in oxygen concentration resulting from algal photosynthesis (oxygen evolution) and microbial respiration (oxygen consumption) using Winkler titrations. Dosing with chlorine as hypochlorite, the form used in this study, confounds the relationship between oxygen change and microbial metabolism because the oxygen content of hypochlorite (OCl) is included in the measurement of total dissolved oxygen concentration within the chamber. This problem was identified after this field experiment had been completed and the resulting data analyzed. Thus, although metabolic measurements were performed as part of this study, the data were considered meaningless for the above reasons and, consequently, were not considered in the analysis process.

Due to problems relating to the measurement of microbial-oxygen dynamics in the presence of chlorine dosing, the focus of data analysis for this experiment was on the response of structural parameters to chlorine exposure. Quantitative samples were obtained from randomly selected diffusers on each sampling

date by scraping all material attached to the clay surface of each diffuser into a collecting container and preserving the material in 3% formalin. In the laboratory, preserved samples were transferred to graduated beakers and agitated using a hand blender to disperse large clumps of algae in order to provide a homogeneous mixture for subsampling. Aliquots of known volumes were removed from each sample and evenly dispersed across coverslips. After air drying, coverslips were placed in a drying oven at 105°C for approximately 48 hr. Following the drying process, coverslips were mounted on glass slides using HYRAX (Custom Research and Development, Auburn, CA). Following the mounting procedure, the remainder of each sample was processed using standard methods (APHA et al. 1985) to obtain ash-free dry mass (AFDM), which was used to estimate total biomass on the substrates.

Cell counts were performed on mounted samples at 1000x on a light microscope using oil immersion. Standard 500 cell counts were performed on each sample to quantify total cell abundance, taxonomic composition, and species evenness. Total algal biovolume was calculated for each sampling by multiply individual species' densities by cell-specific volumes. Volumes were calculated by fitting combinations of simple shapes (e.g., rectangles, triangles) to the cells. Unlike AFDM, biovolume provided a direct estimate of diatom biomass on the substrate. Species evenness was estimated as the number of species identified in the course of a 500 cell count. This measure was

based on the assumption that increased equitability in population density among species present in a sample should result in a greater number of species being encountered during the course of a standard cell count. In contrast, in order to estimate species richness, i.e., the number of species actually present in a sample, asymptotic, as opposed to standardized, cell counts must be employed (e.g., Patrick et al. 1954).

A few diatom populations were abundant in two or more of the streams considered in this experiment. Responses of these dominant populations were analyzed separately in terms of cell density to provide a population-level estimate of differences in ecosystem sensitivity to stress.

Data Analysis. All statistical analyses were performed using the SAS statistical package (SAS Institute 1985). Baseline data collected from each stream just prior to dosing was compared using analysis of variance (ANOVA) followed by multiple comparisons to detect a priori differences in periphyton assemblages among streams.

The response of individual parameters to increased chlorine dosing (i.e., concentration in the flasks) was statistically evaluated using polynomial regression analysis (Kleinbaum and Kupper 1978). This procedure was used to: 1) identify statistically-significant, quantitative relationships between chlorine concentration and periphyton parameters; 2) assess differences in the nature (e.g., linear versus quadratic) of periphyton responses to chlorine among streams; 3) attempt to

find a common dose-response relationship for different parameters in each stream. Two methods were used to compare responses of each parameter among streams. In instances where a significant relationship between a response parameter and chlorine concentration was detected, an EC20 (i.e., concentration at which a 20% change in a response parameter was predicted) was calculated. When identical statistical response models were significant for the same parameter among all streams, dummy variable analysis (Kleinbaum and Kupper 1978) was used to detect statistically significant differences in the slope of response curves among streams for a given parameter. The calculation of EC values is a standard procedure in ecotoxicology, but is dependent upon both the slope and intercept of the dose-response relationship. Consequently, differences in these values among streams may be due to differences in the magnitude of a parameter (e.g., amount of biomass on the substrate) as well as differences in the rate of response to increasing concentration. In contrast, dummy variable analysis allowed for a comparison of regression slopes (i.e., sensitivity of response) among streams independent of the ambient levels of response parameters. In instances where a significant dose-response relationship was not detected, the slope of the relationship was assumed to be zero.

Results

Chlorine dosing and other environmental conditions. Water quality conditions during the study period were relatively distinct in each stream, although certain important similarities

also existed (Table 1). All streams were reasonably oligotrophic in that levels of bioavailable phosphorus and nitrogen were found to be rather low. However, considerable differences in other water quality variables, particularly pH and hardness, were detected among streams. John's Creek, which drained slopes of highly organic soils composed largely of rhododendron detritus, was most dissimilar to the other four streams in that it's waters were relatively soft and mildly acidic. Kimberling Creek, the other stream in an Appalachian drainage basin, was more similar in terms of pH to Bradshaw Creek and Rock Creek, both of which were in the Blue Ridge region, but had the hardest water. Goose Creek had a relatively high pH compared with all other streams but exhibited a hardness similar to the two other streams in the Blue Ridge region, Bradshaw Creek and Goose Creek.

Baseline variation in biological parameters within and among streams. Significant variation in response parameters was observed among substrates in different streams just prior to dosing (Fig. 1). The total amount of organic material accrued on substrates at the end of the 21 day colonization period varied substantially among streams ($p=0.006$, ANOVA F). Considerably more biomass was accrued on substrates placed in Rock Creek compared with all other streams except Bradshaw Creek. The organic matter content of Kimberling Creek samples was almost 10-fold lower than measured in Rock Creek and was the lowest of all five streams.

Differences in the amount of algal biovolume on colonized

substrates among streams was poorly related to differences in total organic content (i.e., AFDM). Algal biovolume was significantly greater on substrates incubated in Kimberling Creek, which had relatively low AFDM, compared with other streams ($p>0.001$, ANOVA F; $p<0.05$, REGWF). Algal biovolume on substrates colonized in Rock Creek was significantly less than that on substrates in all other streams at the commencement of dosing ($p<0.05$, REGWF) despite a relatively high amount of organic matter on substrates in this stream. Baseline variation in algal biovolume among streams prior to dosing was less than that for AFDM (five-fold as compared with almost ten-fold variation).

Stream assemblages varied with respect to their taxonomic diversity and composition. The number of species encountered in the course of standard 500 cell counts was significantly different among streams ($p<0.001$, ANOVA F). The Kimberling Creek assemblage appeared to have the most even algal assemblage, with an average of about 20 species identified per sample. John's Creek was dominated by significantly fewer species than other streams; only 9 species were encountered on average in a sample from this stream. Many species were only dominant in one stream, while a few were abundant in two or more streams during the study period. A list of dominant species is provided in Table 3.

The majority of algal cells in periphyton assemblages in all streams were alive. However, as for other community parameters, the proportion of dead cells varied significantly among streams prior to dosing ($p<0.001$, ANOVA F). Rock Creek had the highest

proportion of dead cells in the algal assemblage, a proportion that was three-fold higher than that in Kimberling Creek, the stream with the lowest proportion of dead cells.

Periphyton responses to chlorine dosing. Dosing with chlorine elicited strong responses from the periphyton assemblage in all five streams tested. Although slight increases in the magnitude of certain parameters was observed at very low levels of chlorine exposure compared with controls, the only significant statistical relationships were those with negative slopes. A negative log-transformed dose-response relationship was deemed most appropriate for modelling both community- and population-level responses based on regression and lack-of-fit tests.

All community-level parameters exhibited negative responses to increased chlorine dosing (Table 2). Scatterplots showing the response of each community parameter to chlorine dosing in each stream are provided in Figures 2-6. Algal biovolume and compositional similarity exhibited a significant and relatively sensitive response to chlorine dosing in all five streams. Algal biovolume was the most sensitive response parameter in Bradshaw and Kimberling Creek and compositional similarity exhibited the most sensitive response in Rock Creek. Ash-free dry mass was the most sensitive response parameter in Goose and John's Creek and exhibited similar sensitivities to biovolume and compositional similarity in Kimberling Creek. However, in contrast to other response parameters, no significant relationship was found between AFDM and chlorine exposure in Rock Creek, where

excessively high amounts of organic material were present on a few substrates (Figure 2). Species evenness and the proportion of dead cells in the assemblages were relatively insensitive to the 7 day chlorine exposure. Significant relationships between species evenness and chlorine exposure were only detected in three streams and, in all cases, less than a 20% effect was observed within the range of chlorine concentrations used. The proportion of dead cells in the periphyton assemblage increased significantly with increased chlorine exposure in all streams, although exposure-related changes were relatively slight compared with most other response parameters.

Dummy variable analysis indicated that stream periphyton assemblages varied significantly in their response to chlorine exposure (Table 2). Total biomass on the substrate responded more sensitively to chlorine exposure in Goose and John's Creek than in Bradshaw and Kimberling Creek. Biomass was insensitive to chlorine exposure in Rock Creek within the range of concentrations used. Algal biovolume exhibited different sensitivities to chlorine exposure in all streams. Rock Creek was most sensitive to chlorine exposure in terms of changes in algal biovolume, while Kimberling Creek was least sensitive. There was no difference in the sensitivity of species evenness to chlorine exposure among the three streams where significant dose-response relationships were detected (Bradshaw, John's, and Rock Creek). However, the Goose Creek assemblage exhibited greater sensitivity to chlorine exposure than other streams in terms of

changes in species composition. Bradshaw Creek was less sensitive to chlorine in terms of this parameter than other streams. The periphyton assemblage in Bradshaw Creek was most sensitive in terms of increases in the proportion of dead cells in response to chlorine exposure while Kimberling Creek was the least sensitive.

The abundance of most dominant populations decreased with increased chlorine dosing in accordance with a logarithmic dose-response model (Table 3). Responses in terms of effect concentrations (EC20) varied dramatically, ranging from less than 0.01 (Synedra minuscula in Bradshaw Creek) to greater than 1.0 for several species. Goose Creek was unique in that several dominant populations exhibited no significant response to chlorine exposure within the 7 day dosing period. In general, there were no other obvious differences among streams in terms of the magnitude of population responses. Dummy variable analysis has not yet been performed on this data set to statistically compare the response of the same population to chlorine exposure in different streams.

Periphyton recovery from chlorine dosing. The logarithmic dose-response model used to detect responses to chlorine exposure was also found to be appropriate for detecting continuing effects of chlorine exposure on periphyton assemblage after 7 days of recovery from dosing. Scatterplots showing the relationship between each community parameter and chlorine dosing regimes imposed earlier in the study are given in Figures 7-11.

Statistical analyses were only performed using data from three streams due to a loss of all substrates in Bradshaw Creek and most substrates Rock Creek as a result of flooding during the recovery period. Several response parameters still exhibited a negative relationship with chlorine dosing regimes even after 7 days of recovery (Table 4). Biomass exhibited noticeable recovery in all three streams sampled after the 7 day recovery period. Although negative relationships between biomass and exposure level were still detected in Goose and John's Creeks, EC20 values were higher than those calculated just after dosing. No relationship between biomass and exposure was detected in Kimberling Creek indicating complete recovery of this parameter within 7 days following exposure. John's Creek periphyton exhibited complete recovery in algal biovolume within 7 days of exposure as indicated by the lack of a significant dose-response relationship for this parameter. Partial recovery of algal biovolume in Kimberling Creek was indicated by an increased EC20 value for this parameter after 7 days of recovery compared with that immediately following exposure. In contrast, the EC20 for algal biovolume in Goose Creek was lower after 7 days of recovery than immediately after exposure, indicating that chlorine exposure continued to elicit a progressive effect on this community parameter. The EC20 for changes in species composition in Goose Creek was also lower after 7 days of recovery compared with immediately following exposure, indicating progressive effects of prior exposure on this parameter as well. The John's

Creek assemblages exhibited full recovery in terms of species composition within 7 days while virtually no recovery in compositional similarity was detected for the Kimberling Creek assemblage. No large change in the relationship between species evenness and exposure level was detected in John's Creek between the beginning and end of the recovery period. The proportion of dead cells in the assemblage returned to control levels in dosed assemblages in Goose Creek after 7 days of recovery while only partial recovery of this parameter was observed in assemblages in John's and Kimberling Creeks.

Dummy variable analysis provided partial support for the hypothesis that minimally-impacted streams may exhibit different resiliences to a novel stressor such as chlorine (Table 4). No differences in resilience among streams was observed for total biomass since Goose and John's Creek continued to be significantly more sensitive to chlorine exposure than Kimberling Creek after 7 days of recovery. Similarly, differences in the resilience of algal biovolume to chlorine exposure among the three streams was similar 7 days after exposure to the responses documented immediately following exposure. Species evenness, which was not strongly affected by dosing in any stream immediately following the exposure period, also exhibited a similar pattern of resilience among streams. Differences in resilience among periphyton assemblages in different streams was indicated by the recovery patterns of compositional similarity and the proportion of dead cells in different assemblages. The

taxonomic composition of the John's Creek periphyton assemblage appeared to recovery more quickly following chlorine exposure than either that of Goose Creek or Kimberling Creek. Whereas the John's Creek assemblage exhibited a moderately sensitive response to chlorine exposure, the taxonomic composition appeared to return to the unimpacted condition within the 7 day recovery period. Dead cell proportions in the Goose Creek assemblage returned to normal more quickly than either the John's Creek or Kimberling Creek assemblages; whereas dead cell proportions increased rather rapidly with chlorine exposure in Goose Creek, these proportions decreased to unimpacted levels within the 7 day recovery period.

Densities of most diatom populations exhibited at least partial recovery during the 7 day period following chlorine exposure (Table 5). Full recovery of some populations in each stream was indicated by fewer significant dose-response relationships after 7 days of recovery compared with the number detected immediately following the exposure period. Densities of all dominant John's Creek populations recovered to control levels on previously exposed substrates within the 7 day recovery period. In contrast, negative dose-response relationships were still detected for most dominant algal populations in Goose Creek and Kimberling Creek after 7 days of recovery. However, most populations in Goose and Kimberling Creeks also exhibited some recovery from chlorine exposure after 7 days as indicated by the higher EC20 values in recovery samples (Table 5) compared with

response samples (Table 3). Densities of two species, Achnanthes lewisiana and A. linearis, both of which were dominant only in Goose Creek, exhibited a lower EC20 after 7 days of recovery compared with that immediately following exposure, indicating a continued response of these populations to chlorine dosing. Dummy variable analysis has not yet been performed on population data to compare dose-response relationships for populations among streams.

Discussion

Organismic responses to chemical stressors are often complex and dependent upon numerous factors including environmental conditions such as temperature and other water quality variables (Rand and Petrocelli 1985), the species under study (Mayer and Ellersieck 1986), and the extent of previous exposure of individuals and populations to the same or other stressors (Cairns et al. 1976, Duncan and Klaverkamp 1983). The response of entire communities to chemicals should also be affected by environmental conditions, the populations present in the community, and the previous history of exposure to the same and other stressors. Thus, as with individual species, it should not be expected that all ecological communities will exhibit identical responses to the same intensity of a stressor. In the present study, we tested the hypothesis that different communities exhibit equivalent responses to the same stressor using diatom communities in reference ecosystems located in the same geographical region. These selection criteria allowed for a

relatively conservative test of the null hypothesis of no difference for the following reasons: 1) the same type of community (diatoms) was tested in each system and modest overlap in the species composition existed among the communities; 2) all ecosystems had a similar history of anthropogenic disturbance; specifically, none of the systems should have experienced previous exposure to chlorine; 3) all ecosystems were geographically proximate to one another and, thus, were exposed to similar regional environmental conditions. However, several diatom species were found in only one of the five streams and there were noticeable differences in important water quality variables such as temperature and pH (and ammonia). Thus, while the five streams were biologically and chemically similar, they were not identical.

All five periphyton assemblages exhibited a rapid and pronounced response to chlorine exposure. However, significant differences in the sensitivity of the periphyton assemblages in different streams to chlorine were detected. Furthermore, the relative sensitivity of a stream to the stressor was dependent upon the response parameter considered. The Kimberling Creek assemblage generally exhibited the greatest resistance to chlorine exposure. The Goose and John's Creek's assemblages were most sensitive in terms of total biomass response, while the Rock Creek assemblage, which exhibited no significant response to chlorine exposure in terms of total biomass, was most sensitive in terms of decreases in algal biovolume. The Goose Creek diatom

assemblage exhibited no change in species evenness in response to chlorine exposure, but was very sensitive from the standpoint of changes in the composition of the assemblage. The Bradshaw Creek assemblage was sensitive to chlorine in terms of changes in species evenness and increases in the proportion of dead cells on the substrate. Thus, different response parameters exhibited different patterns of sensitivity to chlorine among streams.

Differences in water quality variables among streams was incapable of explaining differences in community sensitivity in part because no stream was consistently determined to be more or less sensitive to chlorine exposure. Lower temperatures have been found to increase the persistence of chlorine once released into the environment (Nacci et al. 1990). The streams used in this study were somewhat different in temperature. However, Bradshaw Creek, which had the highest temperature of all the streams, contained a diatom assemblage that was relatively sensitive to chlorine exposure, while the relatively resistant Kimberling Creek assemblage existed at a somewhat lower water temperature. Ammonia can react with free chlorine to form various chlorinated byproducts such as chloramines (Melzian and Jaworski 1991). These reactions may reduce the short-term toxicity of chlorine by reducing the concentration of free hypochlorite in the environment. Ammonia levels were quite low in all streams used in this study; therefore, chlorine-ammonia interactions probably had little effect on chlorine toxicity. Substantial differences in pH among the five streams were

expected to exert a potentially important influence on the partitioning of chlorine between forms of different reactivity (e.g., hypochlorite vs. molecular chlorine). However, there was no consistent relationship between stream pH and community sensitivity.

The sensitivity of an unimpacted community to a novel stressor may be influenced by the species composition of the assemblage if certain species are inherently more resistant to the chemical than others. It is well documented for higher organisms that even closely related species can vary considerably in their sensitivity to a particular chemical (e.g., Mayer and Ellersieck 1986). Diatoms and other protist species exhibit a broad range of tolerances to environmental perturbations as well (e.g., Lowe 1974), although most such evidence comes from studies of responses to gradients of enrichment. It is highly unlikely that the diatom populations in the reference streams used in this study had any prior exposure to chlorine. Therefore, physiological or genetic adaptation mechanisms should not apply (cf. Cairns and Niederlehner 1989). However, some species are inherently less sensitive to a novel stressor as a result of phenotypic characteristics unrelated to the stressor of interest. For example, the observation that diatoms tend to be more sensitive than other groups of algae to chlorine stress (USEPA 1985) is likely due to metabolic or morphological differences among algae that are unrelated to genetic adaptation to chlorine or other toxicants. Population data from this experiment has not

been sufficiently analyzed to determine the extent of differences in sensitivity among diatom populations. Continued analysis on this and other data sets collected from these reference streams will focus on comparing the response of different populations to the same chemical stressor across all streams. Because some species were found to be abundant in two or more of the reference streams, both intra- and inter-specific comparisons will be performed.

The absolute (e.g., EC20) as well as the relative (e.g., dummy variable analysis) sensitivity of different streams to chlorine exposure was dependent upon the response parameter used to estimate sensitivity. Changes in biomass and biovolume were generally the most sensitive responses to exposure while changes in species evenness and increases in the proportion of dead cells in the assemblage were least sensitive. A previous study of chlorine effects on aquatic microbial communities (Pratt et al. 1988) also found that nontaxonomic measures of algal responses (e.g., chl a biomass) were more sensitive to exposure than taxonomic changes in either the algal (i.e., number of algal genera) or protozoan (i.e., number of protozoan species) assemblage. These findings contrast with numerous studies that have found taxonomic indicators (e.g., species richness) to be superior indicators of stream condition with regard to anthropogenic impacts in general (see Patrick 1978). Streams are erosional habitats and, consequently, periphyton mats growing on the stream bottom are susceptible to removal as a result of shear

stresses (Silvester and Sleigh 1985). Exposure to chlorine or other toxic chemicals likely elicits an immediate physiological response from algal cells. Metabolic impairments may affect, among other processes, the ability of algal cells to adhere to the substrate (e.g., decreased mucilage production). Additionally, changes in the metabolic activity of algal cells that affect internal chemistry and storage products can result in increased buoyancy and disattachment from the substrate. Thus, various mechanisms exist whereby chemical exposure may result in a rapid loss of algal biomass from the substrate. The biomass reduction elicited by chlorine may reduce the utility of dead cell proportion as an indicator of chemical impact in two ways: 1) dead cells may be removed from the substrate as portions of the mat are dislodged; 2) physiologically impaired cells may be dislodged from the substrate before they die. Noticeable changes in the taxonomic composition of the assemblage are probably more dependent upon persistent changes in the relative performance of different populations (e.g., differential rates of cell division). Differences in population sensitivities to chlorine were suggested by interspecific differences in EC20 values. However, the effects of differential performance require several cell generations (e.g., weeks) to be detected. Persistent impact over months or years may result in even greater changes in taxonomic composition as resistant species disperse into the ecosystem from outside and displace indigenous populations. However, the process of species accrual between ecosystems

appears to be a slow process (McCormick and Cairns 1992) and would not affect community responses to short-term exposure to stress. Thus, while taxonomic measures may provide a more reliable indication of the extent of chronic or repeated impact, nontaxonomic changes such as biovolume may respond more quickly and thus be more sensitive to acute stress.

With few exceptions (e.g., pesticides), water quality criteria are largely determined on the basis of laboratory exposure regimes. Although the need for field validation of laboratory predictions has been expressed repeatedly (National Research Council 1981, Cairns 1983, Kimball and Levins 1985, Cairns 1990), regulatory decisions continue to be made in the absence of information on chemical effects in real ecosystems. The findings of the present study indicate that natural ecosystems vary in their sensitivity to chemical impacts and that this variation may result from biological as well as chemical differences among systems. Variation in biological responses detected in the present study probably represent a conservative estimate of true variability in response among aquatic ecosystems because streams were selected on the basis of their similarity in geographical location and disturbance history. Increased understanding of the patterns of natural variability in response to anthropogenic impacts and the factors determining ecosystem sensitivity to human disturbance can be used to increase the scientific basis upon which water quality criteria are derived by providing information on the threshold responses of ecosystems in

addition to those of individual species.

Table 1 - Water chemistry parameters measured in the five streams during the chlorine study. Values are means (± 1 standard error) of measurements taken on three dates during the study period. BD - concentration below detection limits for standard methods used.

Parameter	Bradshaw	Goose	John's	Kimberling	Rock
Conductivity (umhos/cm)	79.25 (±5.44)	43.75 (±15.16)	285.75 (±245.7)	276.25 (±105.5)	143.50 (±105.6)
Hardness (mg/L CaCO3)	41.75 (±3.47)	26.75 (±2.56)	7.00 (±0.58)	81.00 (±7.05)	27.75 (±6.43)
pH units	7.16 (±.07)	8.09 (±.14)	6.11 (±.08)	7.10 (±.03)	7.44 (±.08)
Temperature (°C)	19.4 (±1.0)	17.6 (±1.9)	14.7 (±0.8)	15.4 (±0.3)	15.7 (±0.9)
Ammonia (ug/L)	3.7 (±3.67)	9.1 (±4.56)	BD	5.1 (±5.07)	BD
Nitrate (ug/L)	343.5 (±21.8)	951.2 (±332.3)	21.3 (±21.3)	308.5 (±34.4)	885.0 (±221.7)
Nitrite (ug/L)	BD	BD	BD	BD	BD
Orthophosphate (ug/L)	0	2.7 (±2.7)	0	0	5.9 (±3.5)
Silica (ug/L)	5.5 (±0.1)	11.8 (±2.8)	7.1 (±4.7)	6.3 (±0.3)	13.1 (±0.9)

Table 2 - Response of community parameters in each stream to chlorine dosing. The EC20 for each response is provided with the significance of the regression in parentheses below. Groupings based on dummy variable analysis are also provided in parentheses as a subscript following the significance value; streams with the same letter were not significantly different ($p>0.05$, dummy variable analysis.

	Stream				
Parameter	Bradshaw	Goose	John's	Kimberling	Rock
Periphyton Biomass	0.256 (0.005b)	0.012 (0.001a)	0.041 (0.003a)	0.651 (0.042b)	ns
Algal Biovolume	0.184 (0.001b)	0.624 (0.003d)	0.422 (0.001c)	0.554 (0.001e)	0.744 (0.001a)
Species Evenness	>1.00 (0.001a)	ns	>1.00 (0.033a)	ns	>1.00 (0.003a)
Compositional Similarity	0.344 (0.001c)	>1.00 (0.016a)	0.623 (0.001b)	0.598 (0.001b)	0.616 (0.001b)
Proportion of Dead Cells	>1.00 (0.001a)	>1.00 (0.001b)	0.812 (0.001b)	0.776 (0.001c)	>1.00 (0.001b)

Table 3 - Response of dominant populations in each stream to chlorine dosing. The EC20 for the population response is provided with the significance of the regression in parentheses below. NA - species were not dominant in a particular stream.

	Stream				
Population	Bradshaw	Goose	John's	Kimberling	Rock
Achnanthes lanceolata	NA	0.272 (0.001)	NA	NA	>1.00 (0.001)
Achnanthes lewisiana	NA	0.498 (0.001)	NA	NA	NA
Achnanthes linearis	0.164 (0.001)	ns	NA	0.674 (0.006)	0.730 (0.001)
Achnanthes marginulata	NA	NA	>1.00 (0.003)	NA	NA
Achnanthes microcephala	0.836 (0.005)	NA	NA	0.333 (0.001)	NA
Achnanthes minutissima	0.223 (0.001)	NA	NA	0.801 (0.001)	NA
Anomoneis vitrea	NA	NA	NA	0.417 (0.001)	NA
Cocconeis placentula	NA	0.411 (0.001)	NA	NA	NA
Cymbella minuta	NA	0.299 (0.012)	NA	>1.00 (0.022)	NA
Cymbella sinuata	NA	ns	NA	NA	NA
Eunotia exigua	NA	ns	0.523 (0.001)	NA	NA

Table 3 (cont.)

Population	Stream				
	Bradshaw	Goose	John's	Kimberling	Rock
Eunotia incisa	NA	ns	0.356 (0.001)	NA	NA
Fragillaria virescens	NA	ns	0.164 (0.001)	NA	NA
Gomphonema tenellum	NA	NA	NA	NA	0.589 (0.001)
Nitzschia dissipata	NA	0.074 (0.001)	NA	NA	NA
Synedra minuscula	0.008 (0.001)	NA	NA	NA	NA
Synedra rumpens	NA	ns	NA	0.190 (0.001)	NA

Table 4 - Recovery of community parameters in each stream from chlorine dosing. The EC20 for each response is provided with the significance of the regression in parentheses below. Groupings based on dummy variable analysis are also provided in parentheses as a subscript following the significance value; streams with the same letter were not significantly different ($p>0.05$, dummy variable analysis. NA - data not available for that stream.

	Stream				
Parameter	Bradshaw	Goose	John's	Kimberling	Rock
Periphyton Biomass	NA	0.253 (0.013a)	0.164 (0.020a)	ns	NA
Algal Biovolume	NA	0.462 (0.001a)	ns	>1.00 (0.001b)	NA
Species Evenness	NA	ns	>1.00 (0.001)	ns	NA
Compositional Similarity	NA	0.578 (0.001a)	ns	0.582 (0.001b)	NA
Proportion of Dead Cells	NA	ns	>1.00 (0.018a)	0.998 (0.002b)	NA

Table 5 - Recovery of dominant populations in each stream from chlorine dosing. The EC20 for the population response is provided with the significance of the regression in parentheses below. NA - data not available, i.e., either data were not available from the stream (Bradshaw Creek) or species were not dominant in a particular stream.

	Stream				
Population	Bradshaw	Goose	John's	Kimberling	Rock
Achnanthes lanceolata	NA	ns	NA	NA	0.362 (0.001)
Achnanthes lewisiana	NA	0.241 (0.001)	NA	NA	NA
Achnanthes linearis	NA	0.341 (0.001)	NA	0.689 (0.001)	0.782 (0.001)
Achnanthes marginulata	NA	NA	ns	NA	NA
Achnanthes microcephala	NA	NA	NA	>1.00 (0.001)	NA
Achnanthes minutissima	NA	NA	NA	>1.00 (0.001)	NA
Anomoneis vitrea	NA	NA	NA	ns	NA
Cocconeis placentula	NA	ns	NA	NA	NA
Cymbella minuta	NA	ns	NA	>1.00 (0.004)	NA

Table 5 (cont.)

Population	Stream				
	Bradshaw	Goose	John's	Kimberling	Rock
Cymbella sinuata	NA	ns	NA	NA	NA
Eunotia exigua	NA	NA	ns	NA	NA
Eunotia incisa	NA	NA	ns	NA	NA
Fragillaria virescens	NA	NA	ns	NA	NA
Gomphonema tenellum	NA	NA	NA	NA	ns
Nitzschia dissipata	NA	1.00 (0.004)	NA	NA	NA
Synedra minuscula	NA	NA	NA	NA	NA
Synedra rumpens	NA	NA	NA	0.651 (0.001)	NA

Figure Captions (Chlorine Experiment)

Fig 1 - Baseline levels of response parameters on substrates at the commencement of dosing. Bars show the mean of four replicate substrates and error bars are standard erros of the mean.

Fig 2 - Ash-free dry mass (mg/cm^2) on substrates exposed to different concentrations of chlorine for 7 days.

Fig 3 - Diatom biovolume ($um^3/cm^2 \times 10^5$) on substrates exposed to different concentrations of chlorine for 7 days.

Fig 4 - Species evenness (number of species identified in a 500 cell count) of diatom assemblages exposed to different concentrations of chlorine for 7 days.

Fig 5 - Dissimilarity (Euclidean Distance) between diatom assemblages on control substrates and those exposed to different concentrations of chlorine for 7 days.

Fig 6 - Proportion of dead cells in diatom assemblages exposed to different concentrations of chlorine for 7 days.

Fig 7 - Ash-free dry mass (mg/cm^2) on substrates previously exposed to different concentrations of chlorine after 7 days of recovery.

Fig 8 - Diatom biovolume ($um^3/cm^2 \times 10^5$) on substrates previously exposed to different concentrations of chlorine after 7 days of recovery.

Fig 9 - Species evenness (number of species identified in a 500 cell count) of diatom assemblages previously exposed to different concentrations of chlorine after 7 days of recovery.

Fig 10 - Dissimilarity (Euclidean Distance) between diatom assemblages on control substrates and those previously exposed to different concentrations of chlorine after 7 days of recovery.

Fig 11 - Proportion of dead cells in diatom assemblages previously exposed to different concentrations of chlorine after 7 days of recovery.

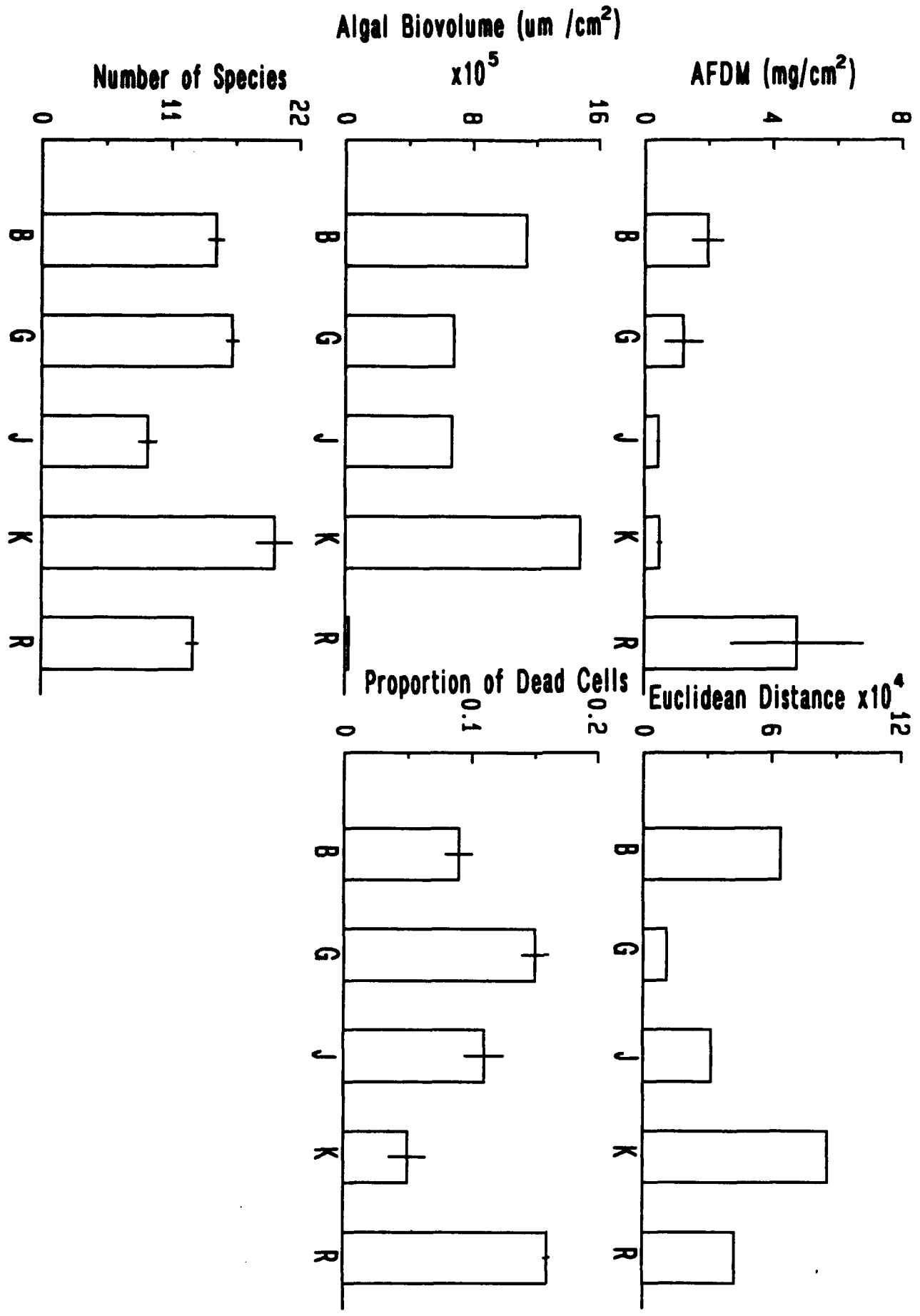

Number of Species
0
11
22
B
G
J
K
R
Algal Biovolume (um /cm²)
x10⁵
0
8
16
B
G
J
K
R
AFDM (mg/cm²)
0
4
8
B
G
J
K
R
Proportion of Dead Cells
0
0.1
0.2
B
G
J
K
R
Euclidean Distance x10⁴
0
6
12
B
G
J
K
R

FIG 1

FIG 2

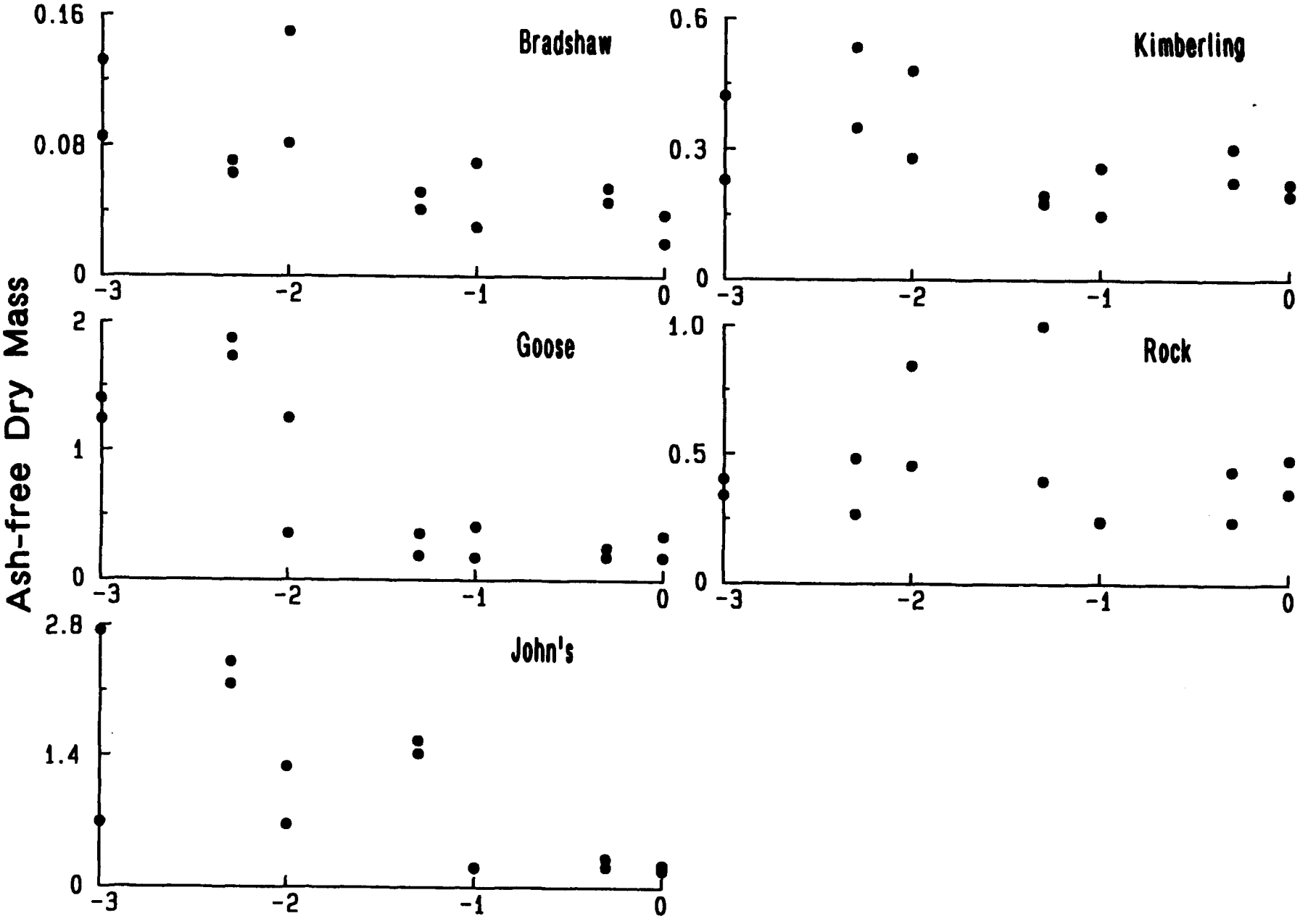

Bradshaw
Kimberling
Goose
Rock
John's
Ash-free Dry Mass
Log Concentration
0.16
0.08
0
0.6
0.3
2
1
1.0
0.5
2.8
1.4
-3
-2
-1
0

FIG 3

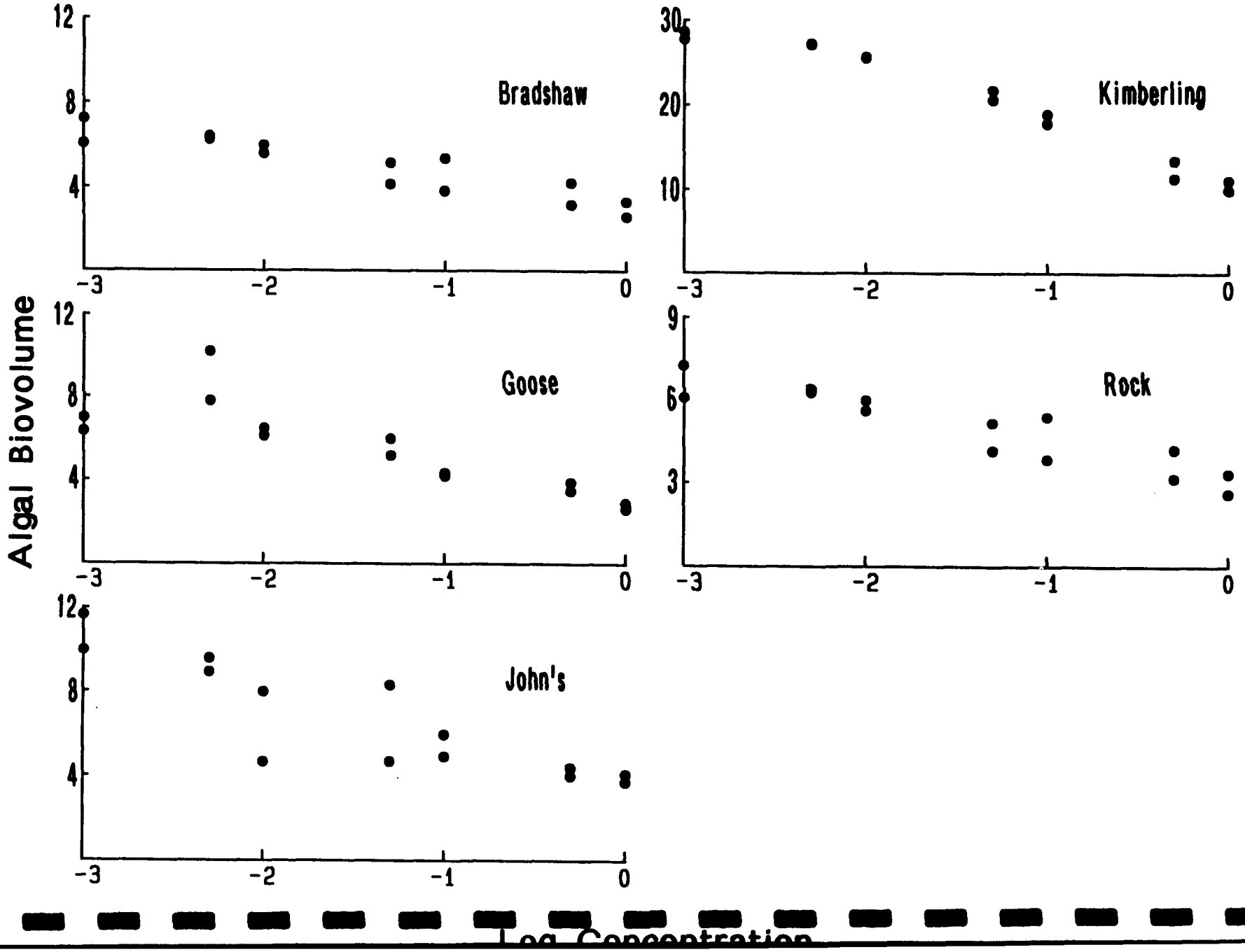

Algal Biovolume
Bradshaw
Kimberling
Goose
Rock
John's
12
8
4
30
20
10
9
6
3
-3
-2
-1
0
Log Concentration

FIG 4

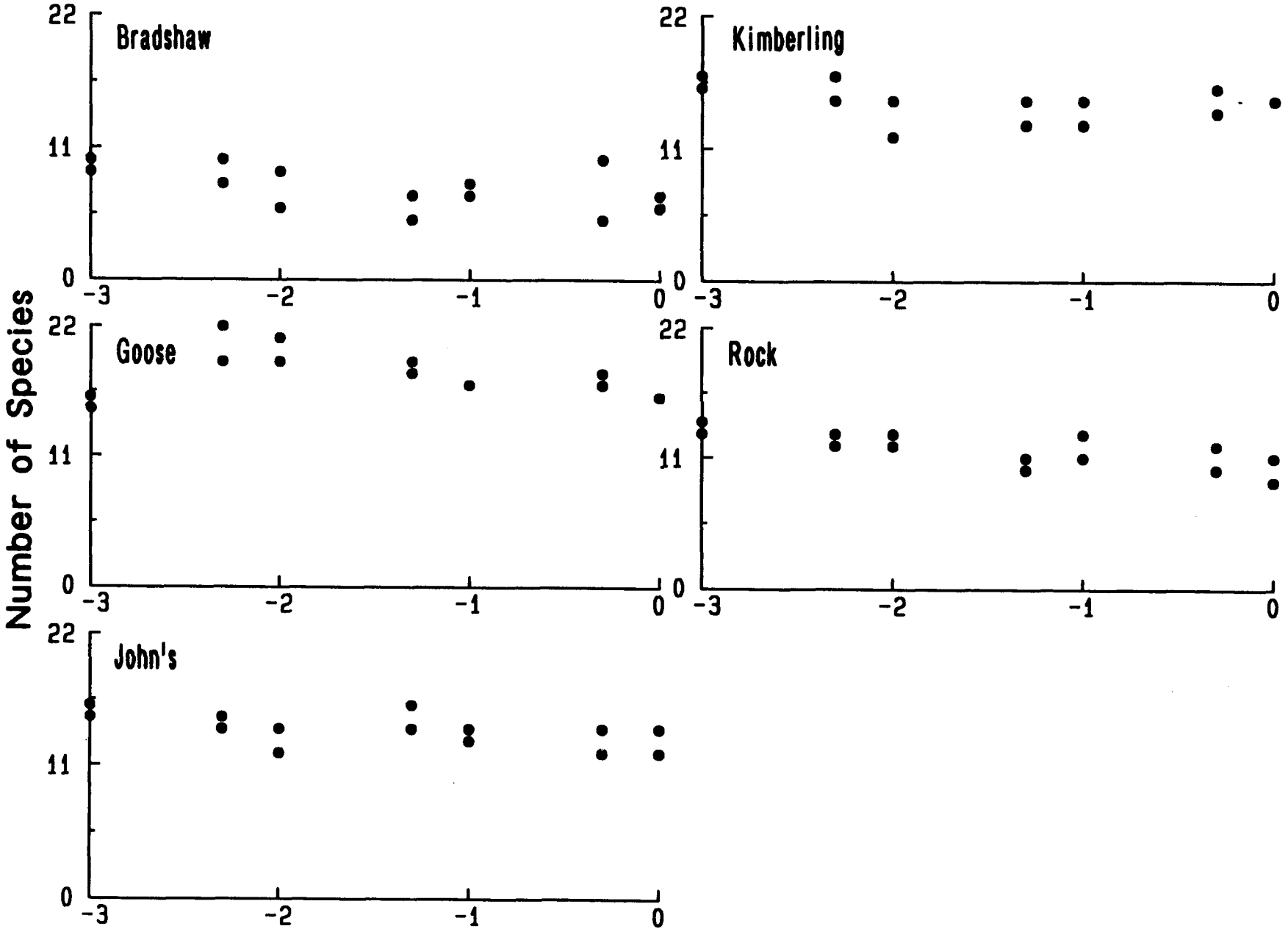

Bradshaw
Kimberling
Goose
Rock
John's
22
11
0
-3
-2
-1
0
Number of Species
log Concentration

FIG 5

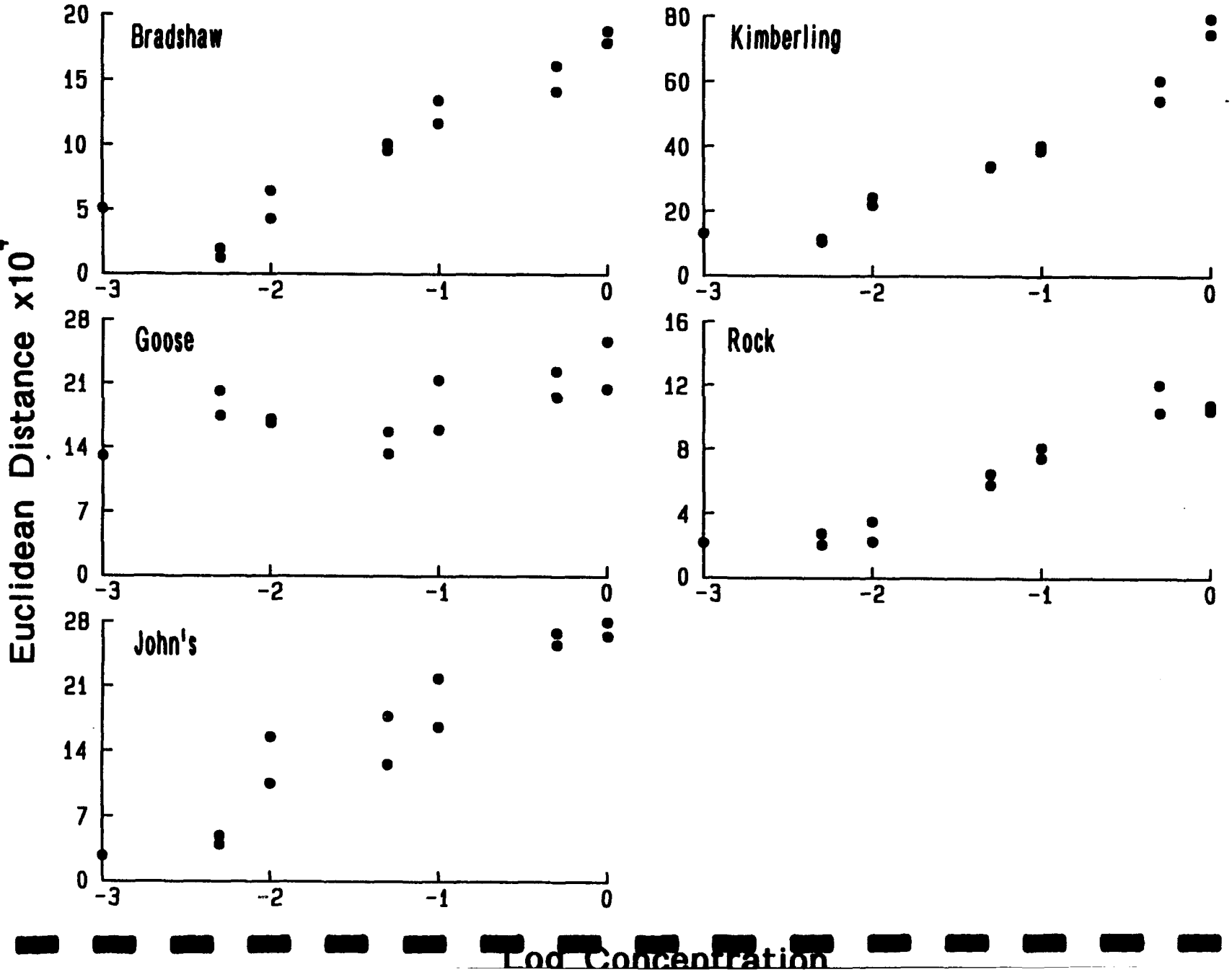

Euclidean Distance x10^4
Bradshaw
Kimberling
Goose
Rock
John's
Log Concentration

FIG 6

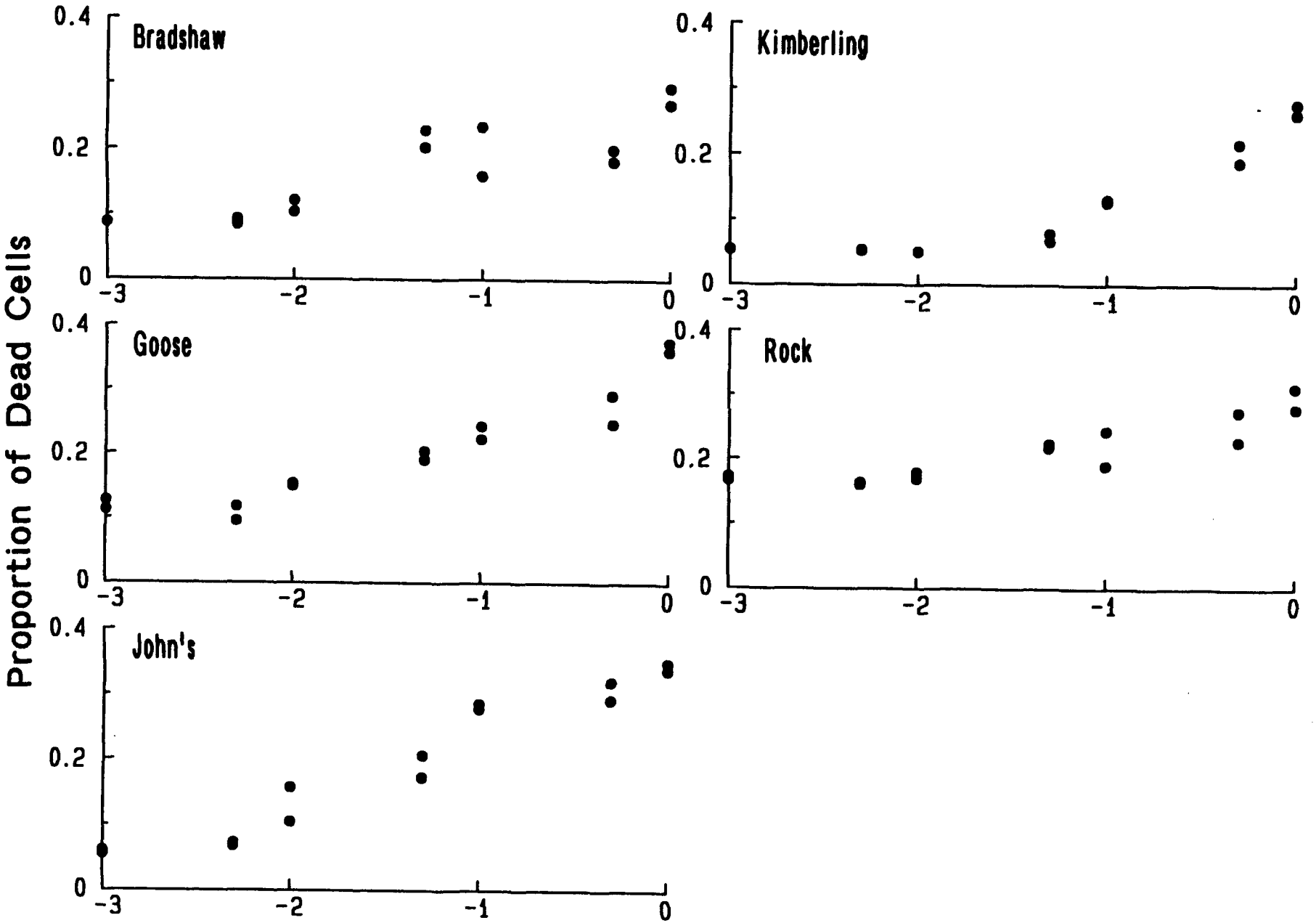

Bradshaw
Kimberling
Goose
Rock
John's
0.4
0.2
0
-3
-2
-1
0
Proportion of Dead Cells
Log Concentration

FIG 7

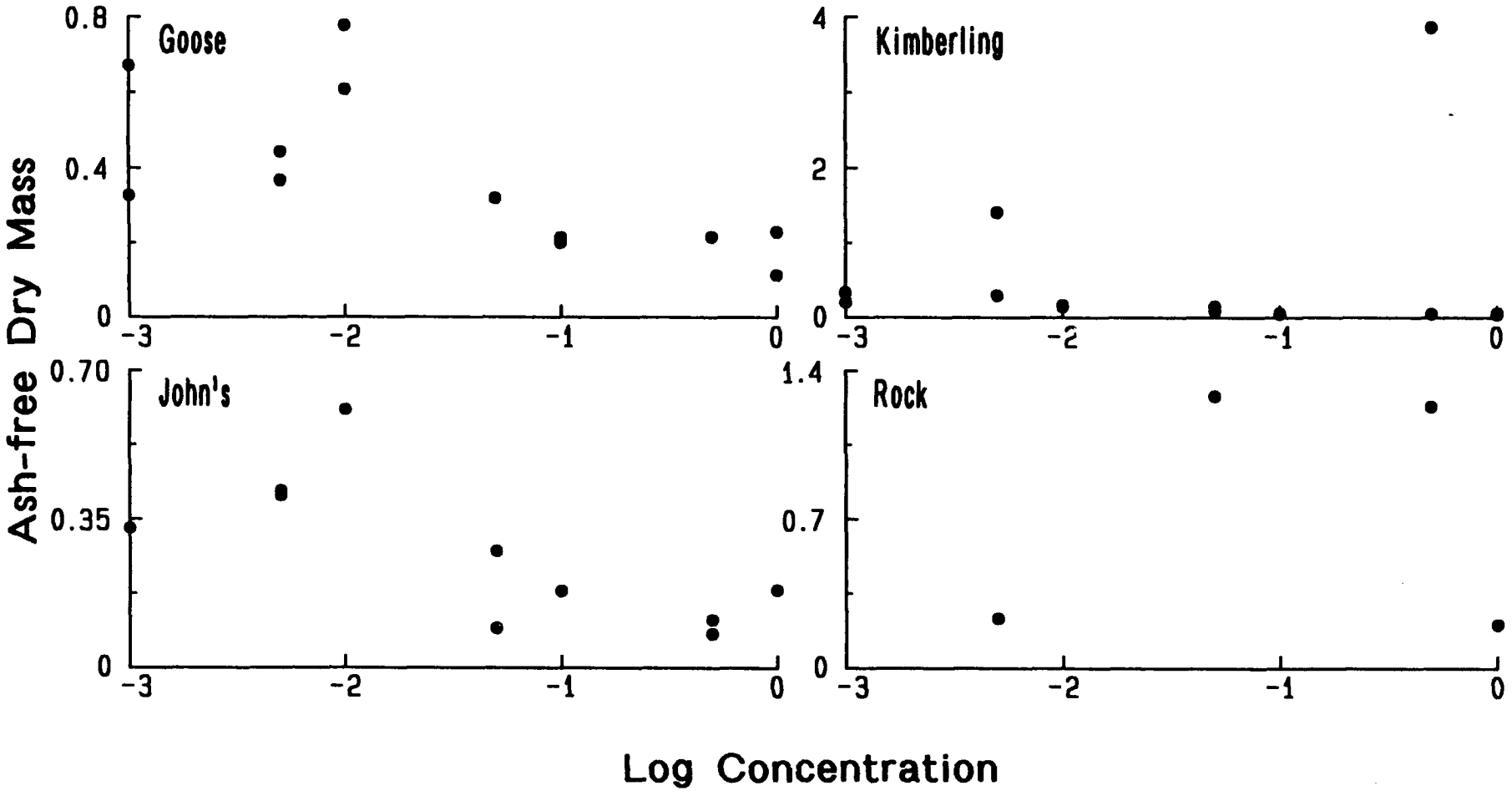

Goose
Kimberling
John's
Rock
0.8
0.4
0
4
2
0
0.70
0.35
0
1.4
0.7
0
-3
-2
-1
0
Ash-free Dry Mass
Log Concentration

FIG 8

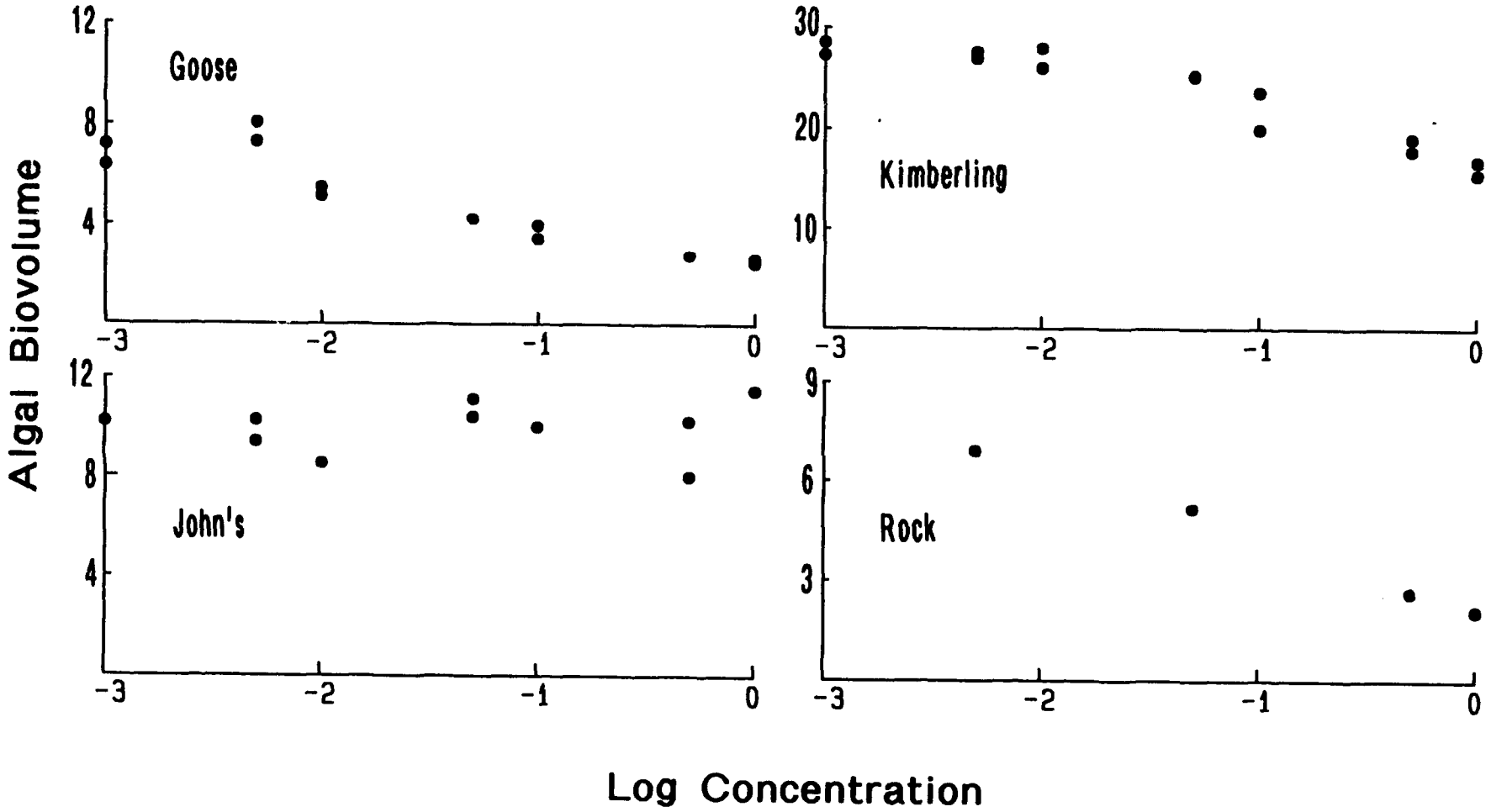

Algal Biovolume
Goose
Kimberling
John's
Rock
12
8
4
30
20
10
9
6
3
-3
-2
-1
0
Log Concentration

FIG 9

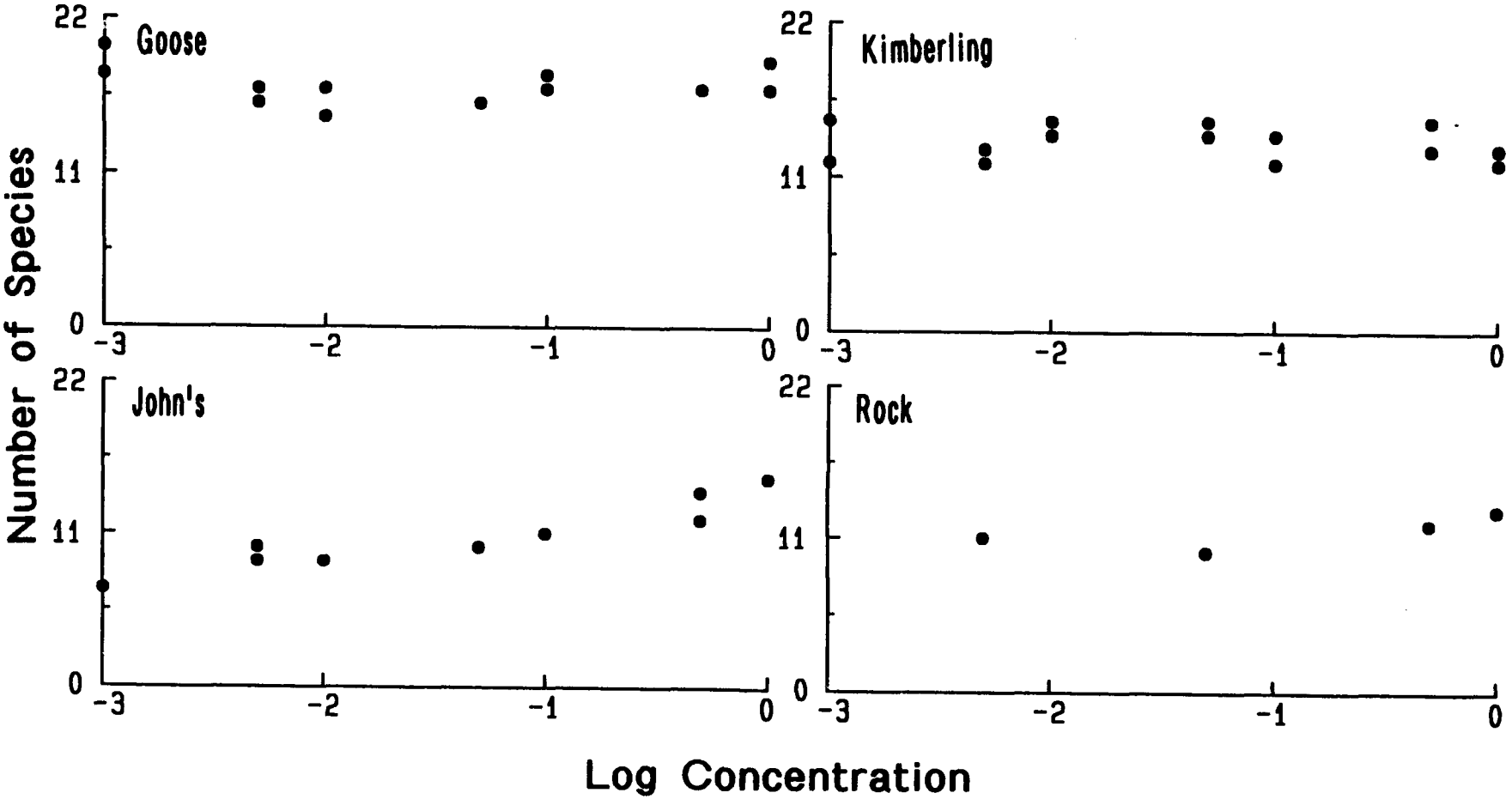

Goose
Kimberling
John's
Rock
Number of Species
Log Concentration
22
11
0
-3
-2
-1
0

FIG 10

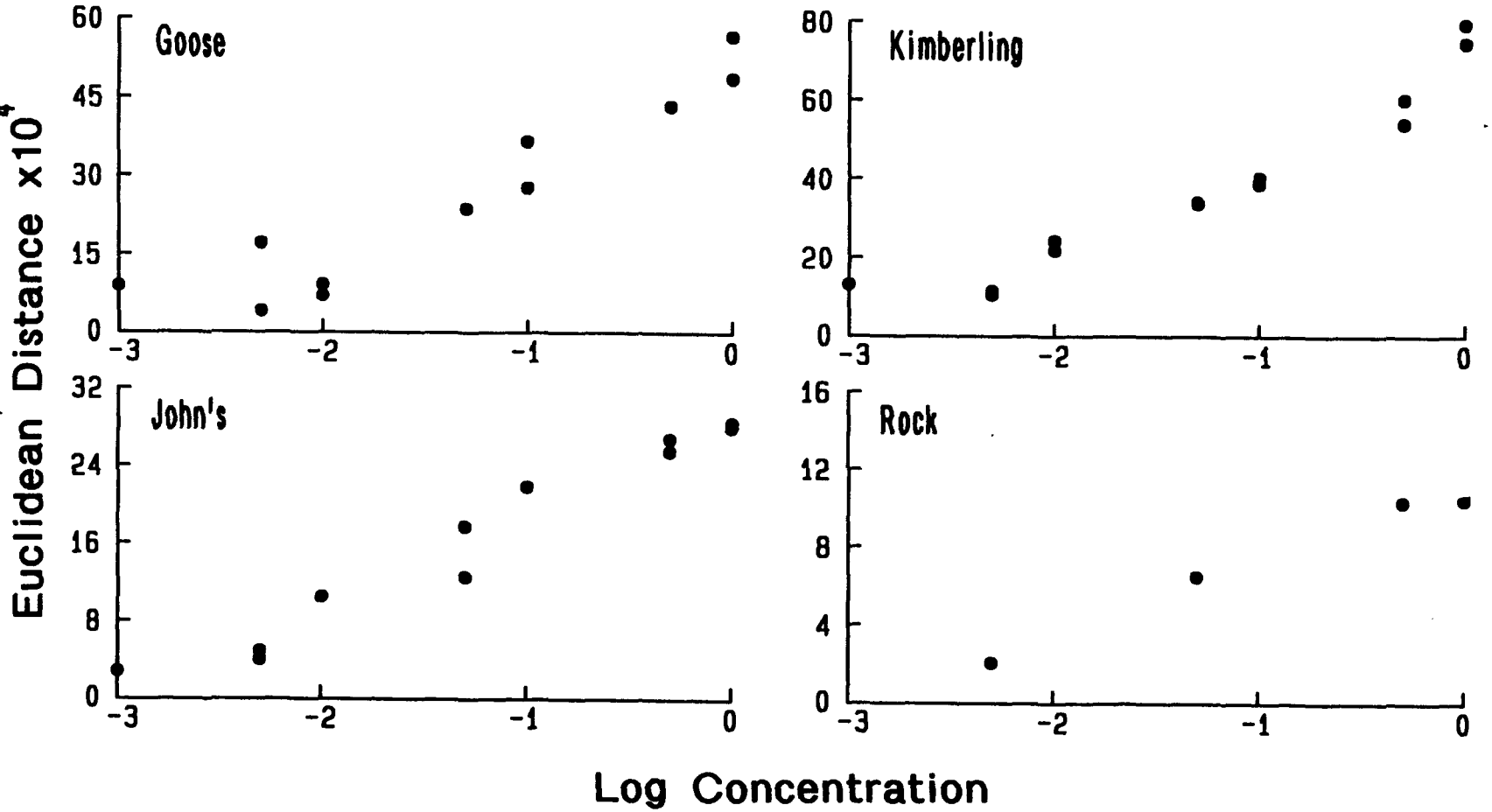
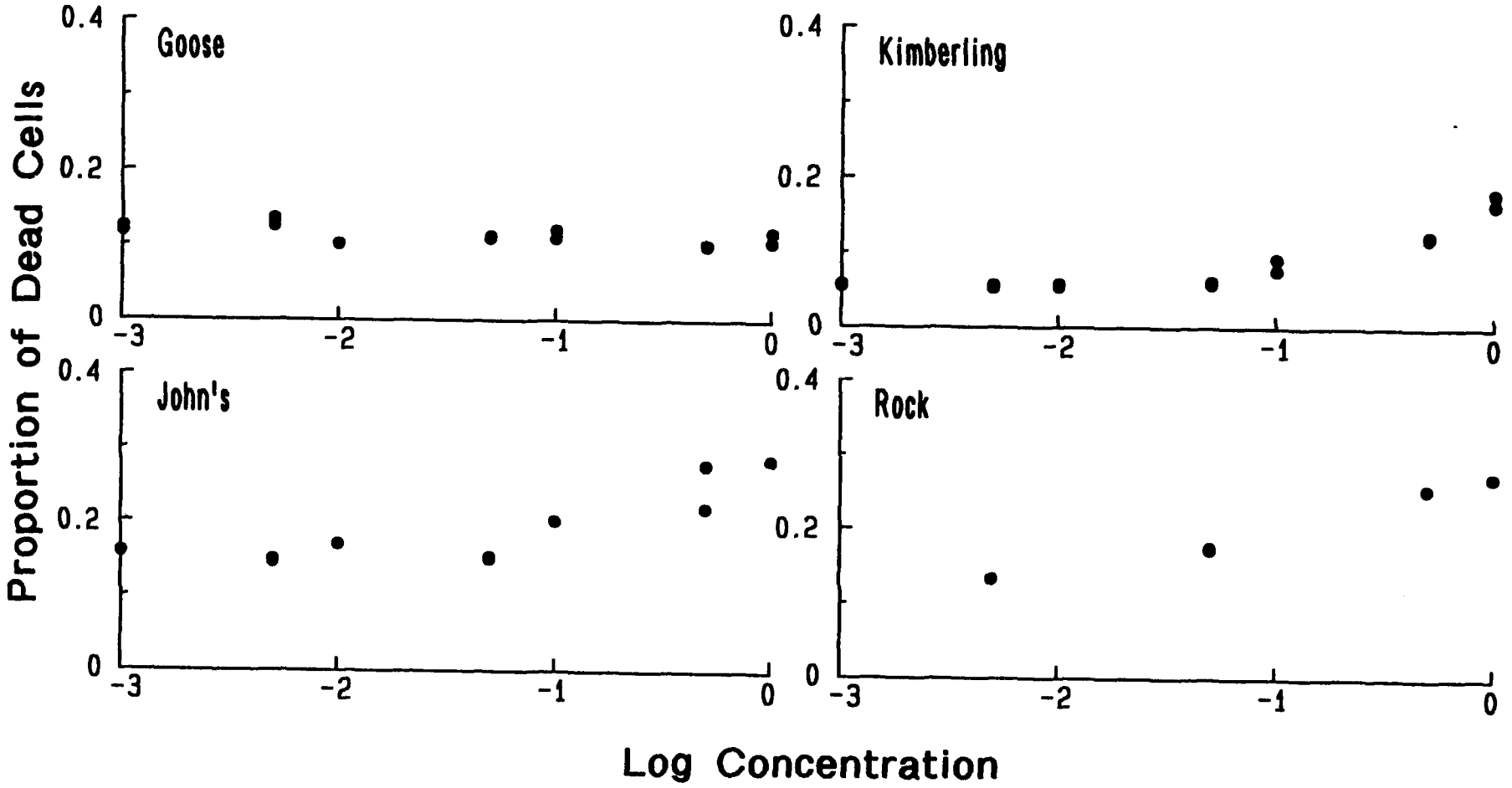

Goose
Kimberling
John's
Rock
Euclidean Distance x10^4
Log Concentration
60
45
30
15
0
80
60
40
20
0
32
24
16
8
0
16
12
8
4
0
-3
-2
-1
0

FIG 11


Goose
Kimberling
John's
Rock
0.4
0.2
0
-3
-2
-1
0
Proportion of Dead Cells
Log Concentration

Section III: Variation in Ecosystem Sensitivity to Acid Stress

Introduction and Objectives

Currently, about 4500 km of streams in the mid-Atlantic region and southeastern United States are acidic as a result of acid mine drainage (Herlihy et al. 1990), and acid precipitation has lowered the pH of surface waters in much of the eastern United States, southeastern Canada, and western Europe (Likens et al. 1979). While the sensitivity of many fish and invertebrate populations to acidification has been well documented (Haines 1981, Burton et al. 1985), comparatively few studies of the effects of lowered pH on stream periphyton communities have been done (e.g., see Stokes 1986 for a review). The studies which have been conducted clearly demonstrate that there is a fairly wide range of pH tolerances among algal taxa.

The toxic effects of acidic inputs on algae in different streams is strongly modified by the physicochemical makeup of the stream. Some chemical attributes (e.g., water hardness) can ameliorate the effects of acidic chemical species, and others (e.g., toxic heavy metals) may interact either synergistically or antagonistically with acids. For example, decreasing pH causes an increase in metal leaching from stream sediments by affecting metal speciation and binding equilibria, and protons may compete for metal binding sites on algal membranes, reducing the uptake of toxic metals (Peterson and Healy 1985). In addition, limited laboratory data suggests that previous exposure of protistan communities to toxic stress (zinc) may lessen later responses to

decreased pH (Niederlehner and Cairns 1993). Thus, in addition to stream chemistry, community composition and adaption in the form of genetic selection and/or physiological acclimation may influence protistan community sensitivity to pH stress. However, the degree to which natural periphyton communities vary in their sensitivities to stress and the importance of the different mechanisms controlling this variation has not yet been experimentally investigated.

The purpose of this experiment was to examine the differences in periphyton responses to introduced sulfuric acid in five unimpacted streams in southwestern Virginia. The same streams investigated in the chlorine experiment (described above) were used in this in situ manipulation. Besides assessing variability in periphyton response to pH at the community level in natural systems, this study served as a prelude to future manipulations with sulfuric acid in both undisturbed and impacted streams within the same biogeographic regions. Taxonomic analysis of the periphyton communities sampled during this investigation are currently in progress. Only the preliminary results of this study are presented below.

Methods

The methods and experimental design employed in this study were the same as those used in the chlorine experiment described above (pp. 11 18), with a few exceptions. In the sulfuric acid experiment the filter-sterilized water that was added to the diffusers prior to the colonization period was replaced with one

of the following concentrations of sulfuric acid at the beginning of the seven-day exposure phase: 0, 0.2, 0.6, 1.0, 2.0, 6.0, or 10.0 normal acid.

The degree of acidification or pH exposure was determined in two ways. First, the flux of sulfuric acid across the terra-cotta tiles was determined by calculating the sulfuric acid concentration both in the stock solutions used to fill the diffusers at the beginning of the exposure phase and in the solutions remaining in the diffusers after seven days of acid leaching. Titrations with sodium hydroxide, standardized with potassium hydrogen phthalate, were used to determine acid concentrations (APHA et al. 1989). Second, pH within the periphyton mat on the tiles was measured on day four of the exposure phase using a modified, flat surface Ag/AgCl electrode (Fisher Scientific). Polyurethane foam (2mm thick) was stretched over the flat electrode surface and held in place against the body of the electrode with rubber bands. This foam covering served to minimize mucilage "poisoning" of the probe. In addition, the foam served to seal the probe to the tile, thereby reducing stream flow over the electrode and enabling stable pH readings to be obtained. The probe was frequently washed with mild detergent, as suggested by the manufacturer, and restandardized to further reduce the likelihood of electrode "poisoning". pH was measured in the center of all substrates present, including those allocated for the recovery phase.

Preliminary Results

Sulfuric acid dosing and other environmental conditions. The physicochemical attributes of the five streams investigated in this study are summarized in Table 1. The entire set of experimental substrates in two streams, Bradshaw Creek and Goose Creek, were lost during heavy spates before baseline periphyton communities were sampled. The water quality conditions in the remaining streams, John's Creek, Kimberling Creek, and Rock Creek, were fairly similar in a number of ways.

The mean daily water temperatures were approximately 18°C in all three streams; this represented a 2-3°C increase over the stream temperatures during the chlorine experiment, which was conducted during the same time of year (June - July). Levels of bioavailable nitrogen and phosphorus were fairly low and relatively comparable in the three streams studied. However, orthophosphate and nitrate were markedly higher than they were during the hypochlorite dosing experiment. During the sulfuric acid manipulation, Rock Creek was distinctive in that it had the highest levels of orthophosphate and nitrate. Ammonia and nitrite were not detected in John's Creek, Kimberling Creek, or Rock Creek during this experiment.

Water hardness and pH were very similar between the two experiments with the same pattern emerging among the three streams. John's Creek had extremely soft, slightly acidic water. The pH was circumneutral in Kimberling Creek, which had the hardest water of the three streams. Rock Creek had the highest

pH (7.42) and an intermediate hardness (24 mg/L as $CaCO_3$). Conductivity varied among streams during the sulfuric acid experiment and between experiments.

The flux of acid (i.e., protons) across the terra-cotta substrates during the exposure phase (Fig. 1) proved to be a highly significant linear function of the internal sulfuric acid concentration ($p<0.0001$ and $R^2>0.92$) in each of the three streams. An analysis of covariance revealed that significant differences existed between the slopes of the three linear relationships ($p=0.0211$). Thus, the diffusers delivered protons in a replicable manner within a stream, yet there were slight but significant differences in the rates of delivery between streams.

Sulfuric acid diffusing across the substrates caused significant reductions of pH within the attached periphyton mats (Fig. 2). Statistically significant linear relationships ($p<0.0001$; $0.48<R^2<0.65$) existed between the pH measured within the mat and the acid concentration in the diffusers. However, pH is equivalent to the negative logarithm of the hydrogen ion concentration, and $\log_{10}$-transforming the internal acid normality improved the fit of these relationships ($p<0.0001$ and $R^2>0.73$ for all three streams). The slopes of the generated regression lines for the three sites were statistically indistinct (ANCOVA $p=0.4763$), but significant differences existed among the y-intercepts (ANCOVA $p<0.0001$). The flux of protons across the substrates and the pH generated in the periphyton communities were probably uniquely modified by the different physicochemical

attributes of the three streams, indicating a need to utilize mean surface pH as the independent variable rather than nominal substrate concentration in the final statistical analyses of this experiment.

Baseline variation in biological parameters within and among streams. The biomass that had accrued on the toxicant-diffusing substrates just prior to dosing differed among streams (Fig. 3). Baseline community biomass in Kimberling Creek was approximately the same as it had been during the previous experiment; however, the biomass in Rock Creek was slightly lower, and that in John's Creek five-fold higher, than previously.

The baseline communities in Kimberling Creek had the greatest rate of oxygen consumption per unit biomass (Fig. 4). and the rates of oxygen consumption in the other two streams were similar to one another. Net primary productivity was only detectable in Kimberling Creek (Fig. 5). Visually, the baseline periphyton communities in Kimberling Creek were algal dominated, whereas the communities in John's Creek and Rock Creek were laden with fine particulate organic matter and sediments. This may have been responsible for the greater degree of baseline metabolic activity per unit biomass in Kimberling Creek compared to that in John's and Rock Creeks. Analysis of taxonomic parameters, currently in progress, should help to further characterize the nature baseline periphyton communities.

Periphyton Responses to Acidification

The variation in community biomass after seven days of

exposure to sulfuric acid is shown in Figure 6. Although negative trends were apparent in all three streams, the relationships between ash-free dry mass and either the acid concentrations in the diffusers or the $\log_{10}$-transformed acid concentrations were not statistically significant ($p>0.15$). Similarly, there were no significant linear relationship between community oxygen consumption and the sulfuric acid concentration in the toxicant-diffusing substrates (Fig. 7).

The lack of acid-induced biomass or respiration changes may be an interesting finding in light of the fact that treatments no lower than pH=4 are typically employed in artificial stream investigations of the ecotoxicological effects of acidification (e.g., Burton et al. 1985, Maurice et al. 1987). In this study, the pH in the periphyton mats was reduced below 4 in Kimberling Creek and below 3 in John's Creek and Rock Creek.

The analysis of community taxonomic changes resulting from substrate acidification and the community attributes following the recovery phase are in progress. Once completed, four additional measures of community response will be used to better elucidate any community shifts following short term acidification and after a subsequent recovery period.

Table 1 - Water chemistry parameters measured in the five streams during the sulfuric acid experiment. Values are means (± 1 standard error) of measurements taken on five dates during the study period. BD - concentration below detection limits for standard methods used.

Parameter	Bradshaw	Goose	John's	Kimberling	Rock
Conductivity (umhos/cm)	108.0 (±2.74)	62.25 (±0.95)	13.80 (±1.53)	192.60 (±25.51)	43.00 (±1.52)
Hardness (mg/L CaCO3)	57.25 (±2.78)	31.70 (±2.23)	8.60 (±0.73)	82.18 (±5.51)	24.12 (±2.34)
pH units	7.28 (±.03)	8.03 (±.13)	6.56 (±.03)	7.12 (±.06)	7.42 (±.04)
Temperature (°C)	20.2 (±0.6)	19.6 (±0.8)	17.9 (±0.3)	17.6 (±0.4)	17.6 (±0.4)
Ammonia (ug/L)	31.9 (±24.5)	13.7 (±1.2)	BD	BD	BD
Nitrate (ug/L)	235.2 (±71.8)	1089 (±100)	382.0 (±129.8)	703.0 (±221.8)	1142 (±213)
Nitrite (ug/L)	BD	BD	BD	BD	BD
Orthophosphate (ug/L)	42.0 (±24.3)	49.0 (±6.5)	26.1 (±16.4)	11.6 (±9.7)	46.7 (±5.2)
Silica (ug/L)	4.5 (±1.1)	9.2 (±2.7)	3.2 (±0.4)	6.0 (±1.1)	8.8 (±2.0)

Figure Captions (Sulfuric Acid Experiment)

Fig 1 - Acid (i.e., proton) flux from substrates filled with different concentrations of sulfuric acid in three streams. Statistical results of linear regressions are shown in the upper left hand corner of the graphs.

Fig 2 - pH measured in periphyton mats attached to the surface of substrates filled with different concentrations of sulfuric acid in three streams. Statistical results of linear regressions of surface pH on $\log_{10}$-transformed acid concentration are shown in the upper right hand corner of the graphs.

Fig 3 - Baseline levels of periphyton biomass accrued on the toxicant-diffusing substrates at the commencement of dosing. Bars show the mean of four replicate substrates and error bars are standard errors of the mean.

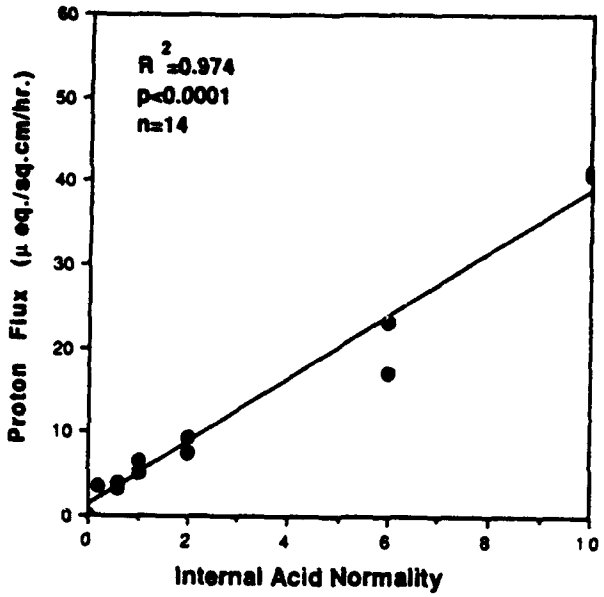
Fig 4 - Baseline oxygen consumption rates of periphyton communities attached to the substrates. Bars show the mean of four replicate substrates and error bars are standard errors of the mean.

Fig 5 - Baseline net primary productivity of the periphyton communities attached to the substrates. Bars show the mean of four replicate substrates and error bars are standard errors of the mean. 'NC' = not calculated because oxygen changes were undetectable.

Fig 6 - Ash-free dry mass (mg/cm^2) on substrates exposed to different concentrations of sulfuric acid for 7 days.

Fig 7 - Oxygen consumption rates of periphyton communities exposed to different concentrations of sulfuric acid for 7 days.

FLUX OF PROTONS ACROSS THE SUBSTRATES
JOHNS CREEK



FLUX OF PROTONS ACROSS THE SUBSTRATES
KIMBERLING CREEK

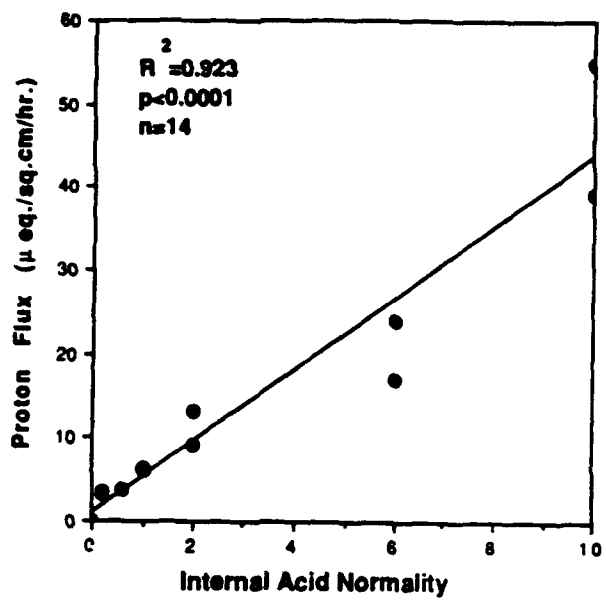
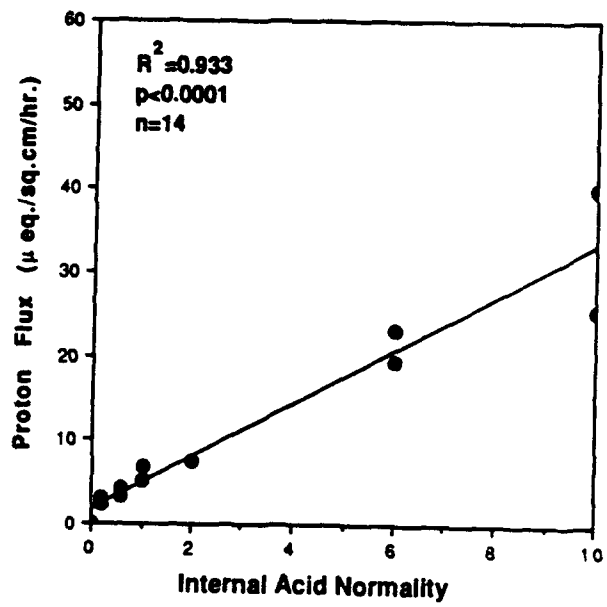
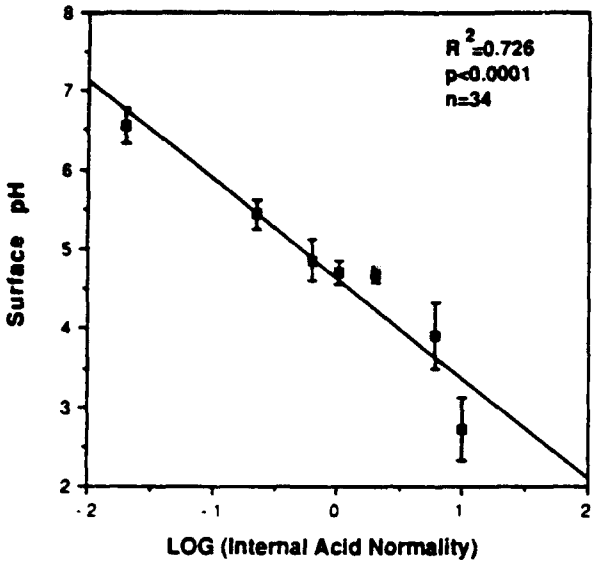


FIG 1

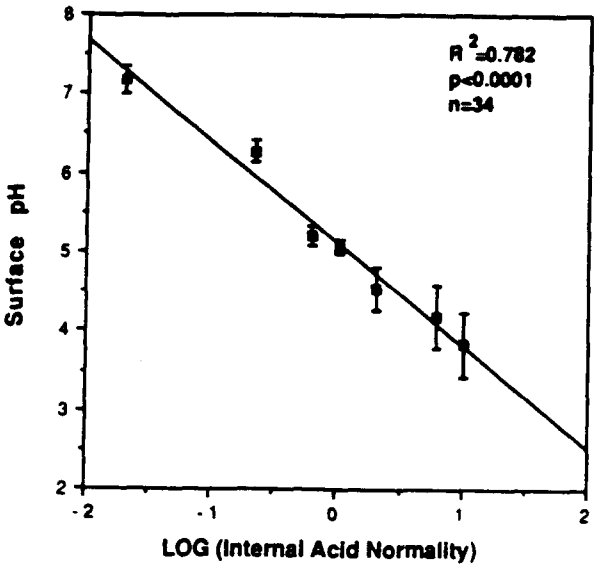
FLUX OF PROTONS ACROSS THE SUBSTRATES
ROCK CREEK



SURFACE pH ON DAY 4 OF THE EXPOSURE WEEK
JOHNS CREEK



SURFACE pH ON DAY 4 OF THE EXPOSURE WEEK
KIMBERLING CREEK



SURFACE pH ON DAY 4 OF THE EXPOSURE WEEK
ROCK CREEK

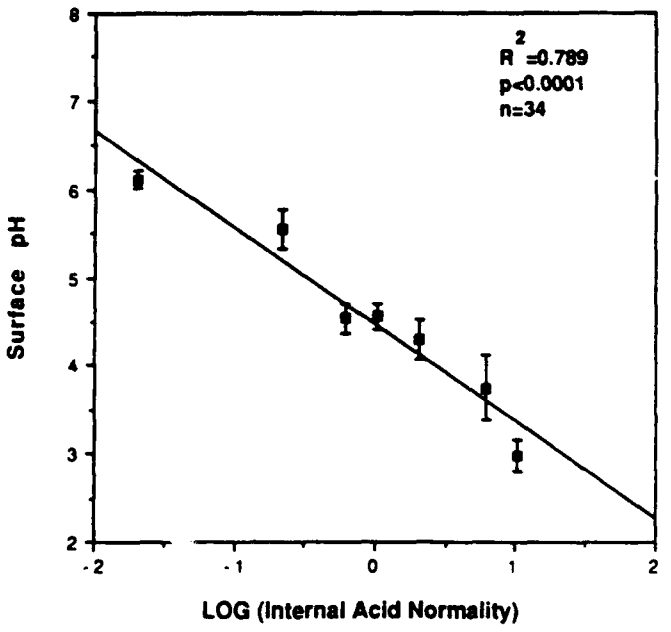


FIG 2

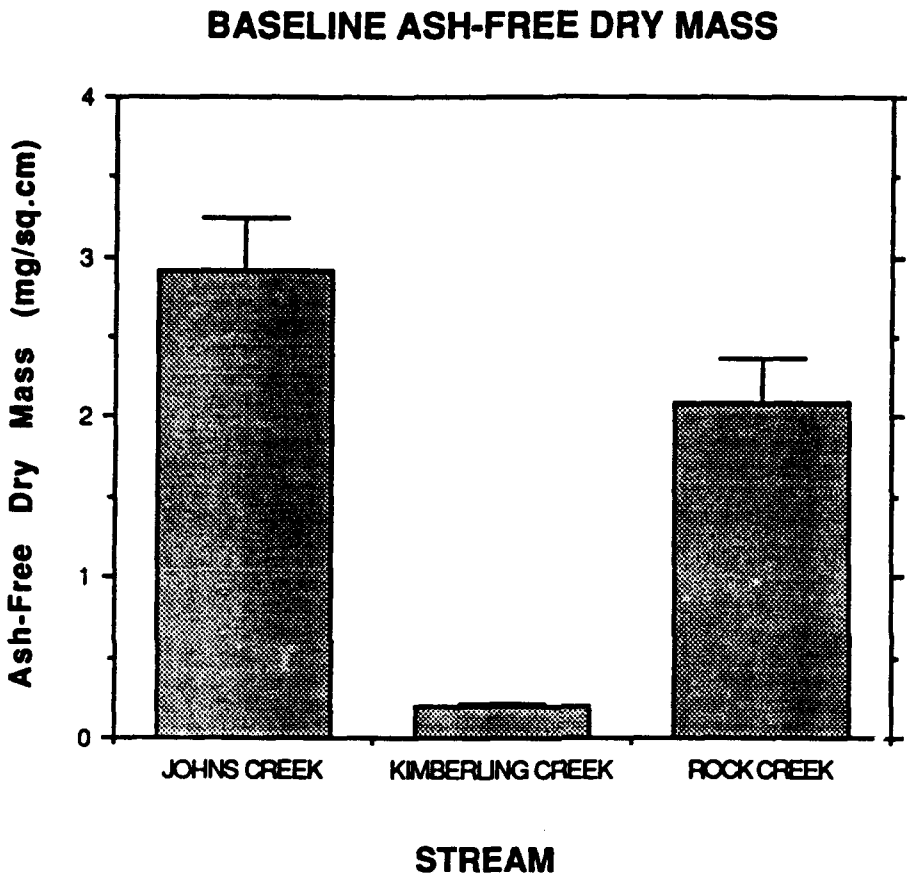

BASELINE ASH-FREE DRY MASS
Ash-Free Dry Mass (mg/sq.cm)
4
3
2
1
0
JOHNS CREEK
KIMBERLING CREEK
ROCK CREEK
STREAM

FIG 3

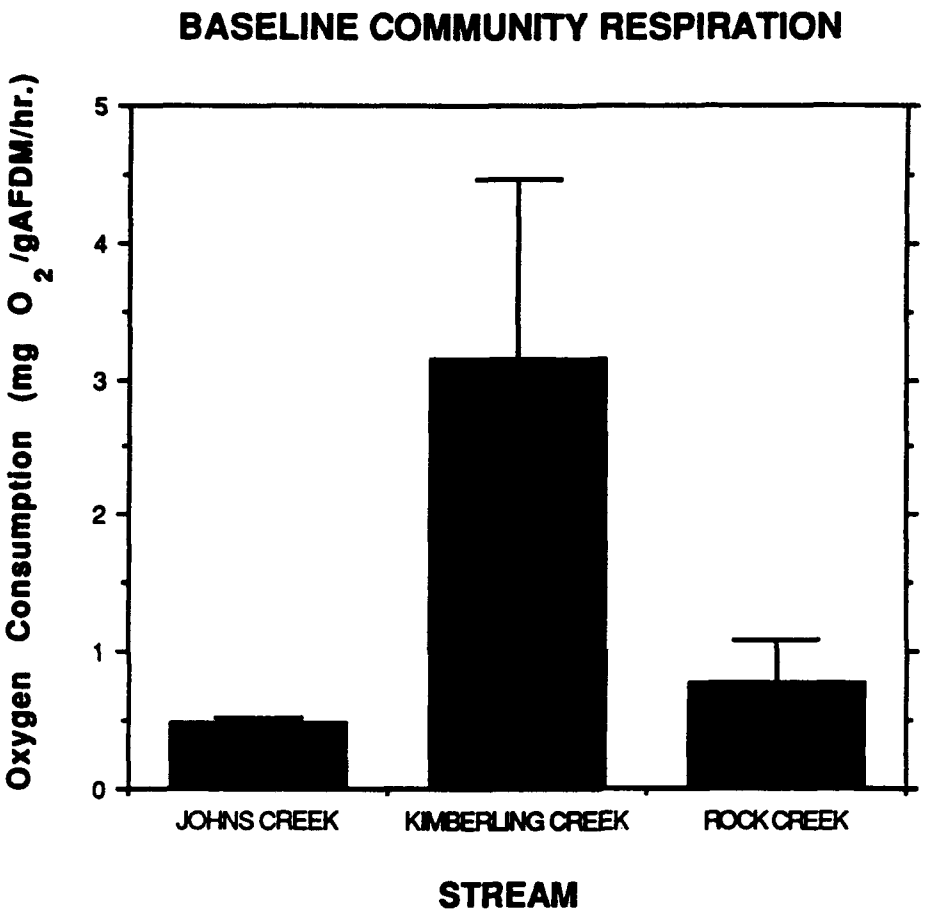

BASELINE COMMUNITY RESPIRATION
Oxygen Consumption (mg O2/gAFDM/hr.)
5
4
3
2
1
0
JOHNS CREEK
KIMBERLING CREEK
ROCK CREEK
STREAM

FIG 4

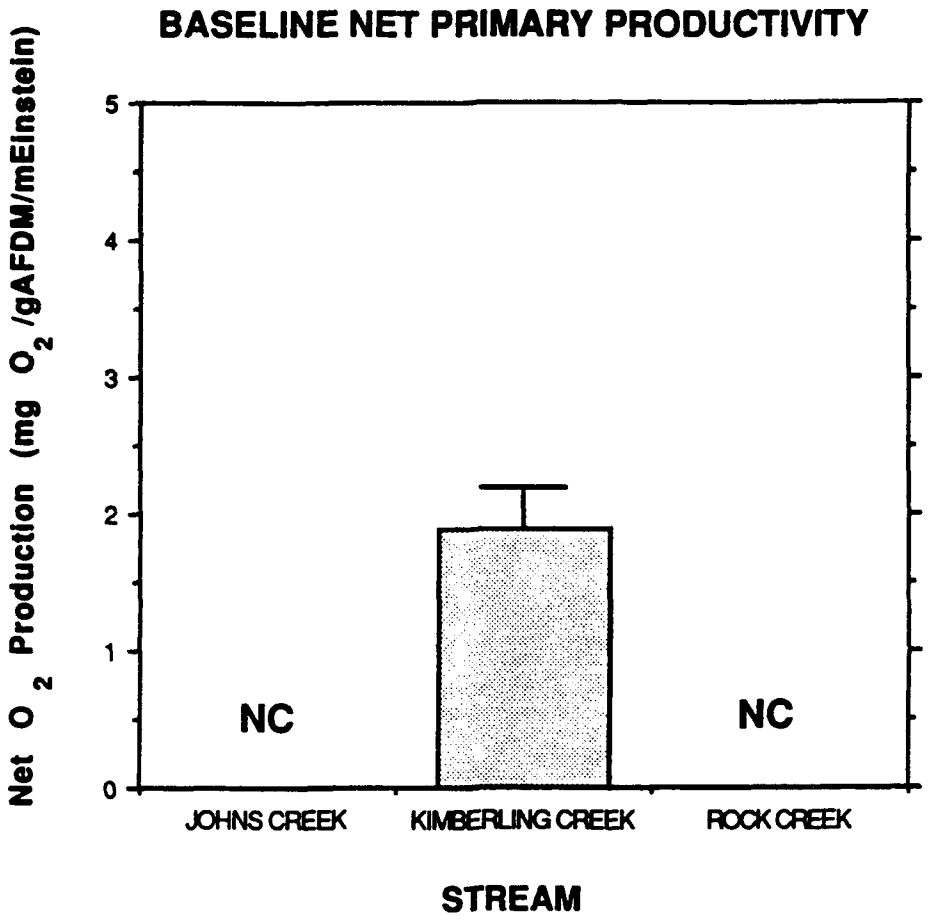
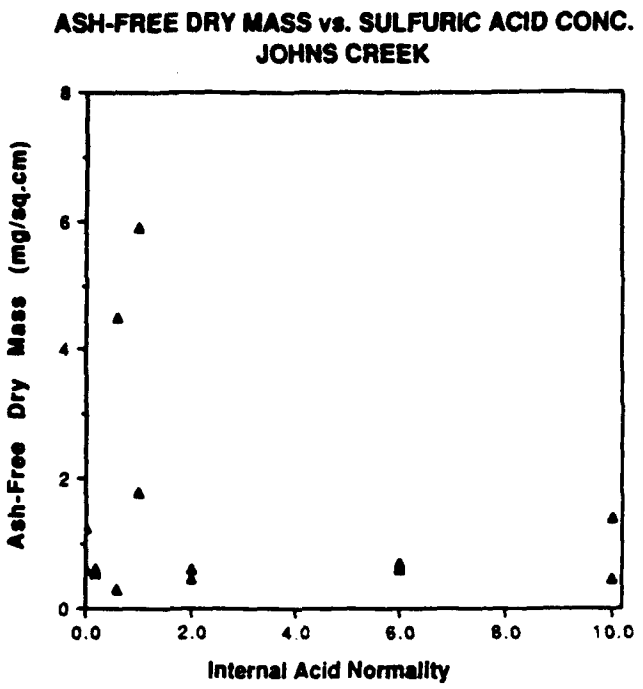

BASELINE NET PRIMARY PRODUCTIVITY
Net O2 Production (mg O2 /gAFDM/mEinstein)
5
4
3
2
1
0
NC
NC
JOHNS CREEK
KIMBERLING CREEK
ROCK CREEK
STREAM

FIG 5


ASH-FREE DRY MASS vs. SULFURIC ACID CONC.
JOHNS CREEK
Ash-Free Dry Mass (mg/sq.cm)
8
6
4
2
0
0.0
2.0
4.0
6.0
8.0
10.0
Internal Acid Normality

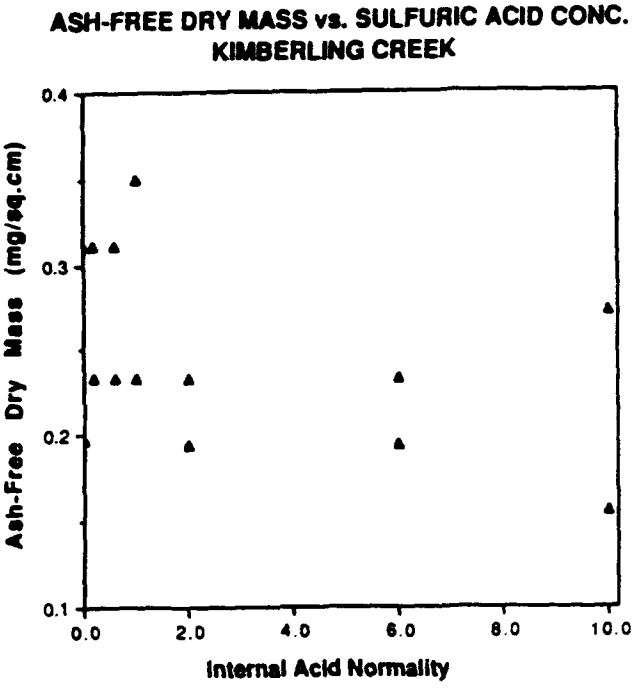
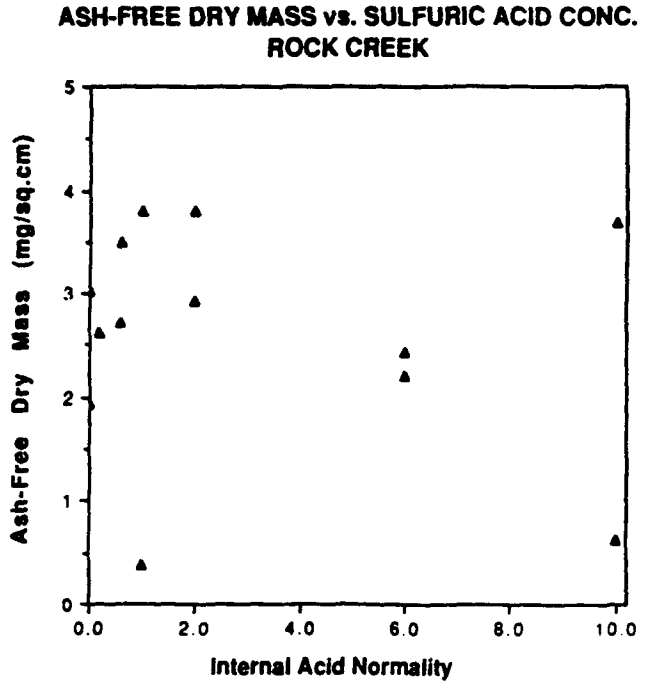
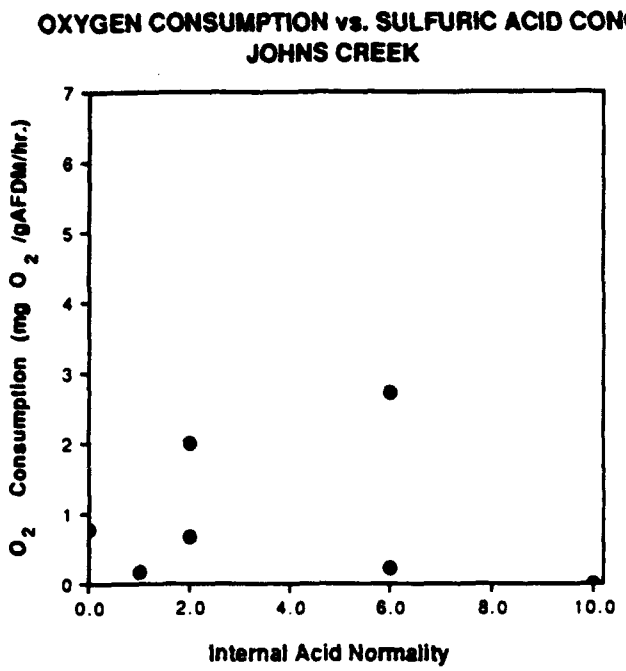

ASH-FREE DRY MASS vs. SULFURIC ACID CONC.
KIMBERLING CREEK
Ash-Free Dry Mass (mg/sq.cm)
0.4
0.3
0.2
0.1
0.0
2.0
4.0
6.0
8.0
10.0
Internal Acid Normality

FIG 6


ASH-FREE DRY MASS vs. SULFURIC ACID CONC.
ROCK CREEK
Ash-Free Dry Mass (mg/sq.cm)
5
4
3
2
1
0
0.0
2.0
4.0
6.0
8.0
10.0
Internal Acid Normality


OXYGEN CONSUMPTION vs. SULFURIC ACID CONC.
JOHNS CREEK
O2 Consumption (mg O2 /gAFDM/hr.)
0
1
2
3
4
5
6
7
0.0
2.0
4.0
6.0
8.0
10.0
Internal Acid Normality

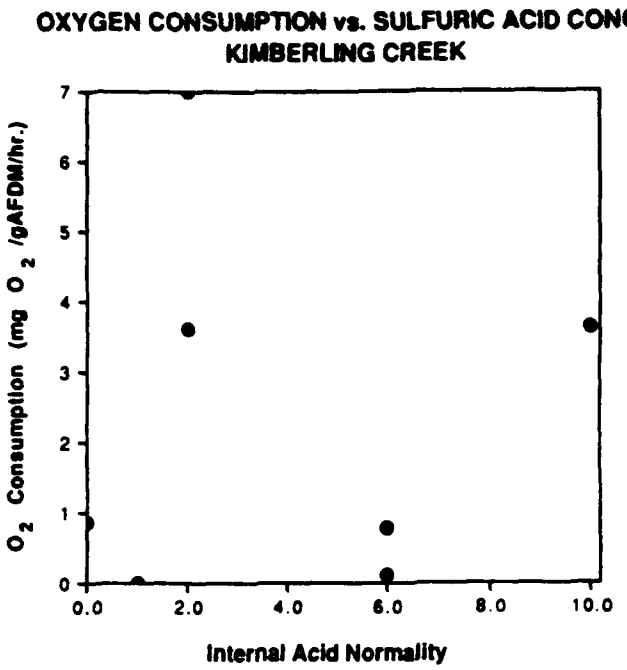
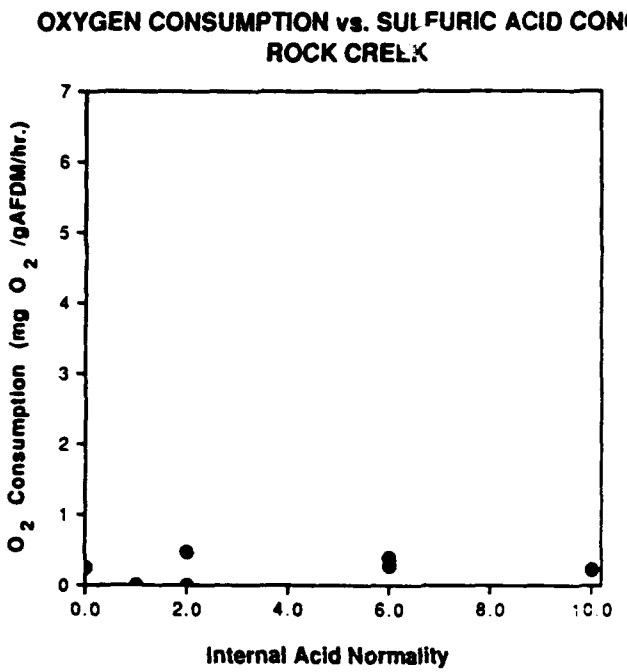

OXYGEN CONSUMPTION vs. SULFURIC ACID CONC.
KIMBERLING CREEK
O2 Consumption (mg O2 /gAFDM/hr.)
0
1
2
3
4
5
6
7
0.0
2.0
4.0
6.0
8.0
10.0
Internal Acid Normality

FIG 7


OXYGEN CONSUMPTION vs. SULFURIC ACID CONC.
ROCK CREEK
O2 Consumption (mg O2 /gAFDM/hr.)
0
1
2
3
4
5
6
7
0.0
2.0
4.0
6.0
8.0
10.0
Internal Acid Normality

Ongoing Research and Future Avenues of Inquiry

Currently, a new chemical pollutant (cupric chloride) is being used as an experimental stressor in a comparative study of the toxicological responses of periphyton communities in five "pristine" and five historically impacted streams. Accurate comparisons of the microbial responses to toxic stress in different streams relies on a sound means of measuring dose. Exposure levels during the previous studies with chlorine and sulfuric acid have been measured either indirectly by calculating toxicant flux or directly using an electrode modified to quantify the chemical environment (e.g., the pH) in the periphyton mat. Measurements of dose obtained by calculation (e.g., toxicant loss from the diffusers) are not as informative as ambient measurements of toxicant concentration. Additionally, methods of directly quantifying pH within the periphyton mat are too labor intensive and time consuming to utilize in a ten-stream field study.

Copper (II) chloride is being used as the experimental stressing agent because: 1) data have been generated which allow the treatment levels (i.e., toxicant concentrations placed in the chemical-diffusing substrates) to be precisely determined in advance (Arnegard 1993), thereby preventing the loss of field time due to treatments being chosen which are either too low or too high to generate informative dose-response relationships; and 2) periphyton bioconcentration of copper is a valid measure of dose for which standardized methods exist (Campbell and Stokes 1985).

Impacted sites have been chosen for this study on the basis of the following criteria: 1) a well documented history of heavy metal impact is present; 2) copper, while inevitably present in all stream sediments, is not a major heavy metal constituent of the sediments; 3) the sites are located close to the research facilities; and 4) permission to access the sites can be obtained from the land owner or discharging industry. Finding five sites which fit these criteria required a broader geographic search than expected. Therefore, two of the previously studied "pristine" sites were replaced with new unimpacted sites to increase the degree to which the study site locations and water quality characteristics of the reference and impacted sites are interspersed.

While the historical impacts are well known for the five disturbed sites, data have been collected to validate these impacts. Samples have been collected which enable three environmental indicators to be calculated: 1) the Riparian, Channel, and Environmental (RCE) Inventory, which primarily assesses the quality of the stream and riparian habitat (Petersen 1992); 2) the Macroinvertebrate Rapid Bioassessment Protocol (RBP II) score, which assesses the impairment of a major biological component of streams ecosystems (USEPA 1989); and 3) an Index of Pollution (IP) for non-residual trace metals, which assesses the heavy metal load on stream sediments (Chester et al. 1985). These three, non-redundant indices are being used as the three axes in a graphical approach to validating the segregation of the

unimpacted streams from the impacted streams. Data for the RCE and RPB II axes have been analyzed and illustrate good segregation. Heavy metals in sediment digests, which are still being analyzed, suggest good segregation between the two classes of streams as well.

In this study, 22 chemical-diffusing substrates per site were filled with filter-sterilized water, pseudorandomized, and secured to pallets anchored to the bottom of each stream, as before. Following a three-week colonization period, periphyton communities were sampled from four substrates in each stream, previously allocated as baseline substrates. Each of the remaining substrates was filled with one of the following concentrations of cupric chloride expressed as gCu^{++}/L (three substrates per concentration): 0, 0.5, 1.0, 2.0, 4.0, or 6.0. These copper concentrations were chosen on the basis of an artificial stream experiment, previously conducted using AFOSR-91-0379 funds (Arnegard, 1993).

At the end of a one-week period of exposure to copper the communities in all streams were sampled in the manner described for the chlorine experiment. Before being preserved, however, aliquots of the sampled communities were removed and washed with a 1.0% EDTA solution on site to remove copper bound to the cell surfaces and abiotically bound copper, leaving only internally bound copper (Campbell and Stokes 1985). These subsamples have been frozen for later analysis of copper bioconcentration in the periphyton mat. The remaining portions of sample were then

preserved for subsequent analysis of community biomass and algal composition.

The primary objective of this experiment is to assess whether or not the sensitivities of the periphyton communities in unimpacted streams to novel copper stress are significantly different from the sensitivities of periphyton communities in the impacted streams to novel copper stress. The measured responses of copper treated communities will be compared between the two classes of streams. Significant differences in the responses will suggest that history of stress is an important modifier of community sensitivity.

Although data analysis for this study was just recently initiated, field observations made during the experimental manipulations suggested that periphyton communities in the impacted communities were more resistant to copper than those in the unimpacted streams. Copper induced a visual loss of pigmentation and biomass from communities in the unimpacted streams; however, little or no visible loss occurred in the impacted streams.

Future lines of inquiry in this project will be modified after all the data collected to date is analyzed this winter. Initially, the previously utilized toxicant stress (i.e., chlorine or sulfuric acid) which elicits the most characteristic responses in unimpacted streams will be delivered to the set of "pristine" and historically impacted streams using the established experimental design. If it is deemed necessary to

improve analytical resolution, additional replicate substrates will be introduced into each stream, and impacted streams in which history of stress plays little or no role in modifying sensitivity to copper stress may be dropped from future experiments or replaced with other impacted sites. Any adaptation to a novel metal stress in historically metal-impacted streams characterized by the current research will be followed up with investigations utilizing unrelated novel stressors. This will be done to investigate the degree to which adaptation to stress is generalizable across different classes of toxic chemicals in stream ecosystems, the ultimate goal of this project.

Literature Cited

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 16th ed. Washington, D.C.

Arnegard, M. E. 1993. Toxicant-Releasing Substrates: A New Method for Delivering Copper to Microbial Communities in situ. Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Master of Science in Biology. 131 pp.

Burton, T. M., R. M. Stanford, and J. W. Allan. 1985. Acidification effects on stream biota and organic matter processing. Can. J. Fish. Aquat. Sci. 42:669-675.

Cairns, J., Jr. 1983. Are single species test alone adequate for estimating hazard? Hydrobiologia 137:271-278.

Cairns, J., Jr. 1990. The prediction, validation, monitoring, and mitigation of anthropogenic effects upon natural systems. Environmental Auditor 2:19-25.

Cairns, J., Jr., D. I. Messenger, and W. F. Calhoun. 1976. Invertebrate response to thermal shock following exposure to acutely sublethal concentrations of chemicals. Arch. Hydrobiol. 77:164-175.

Cairns, J., Jr. and B. R. Niederlehner. 1991. Final Technical Report to the Air Force Office of Scientific Research. Grant AFOSR-88-0263

Cairns, J., Jr., B. R. Niederlehner, and J. R. Pratt. 1991. Evaluation of joint toxicity of chlorine and ammonia to aquatic communities. Aquat. Toxicol. 16:87-100.

Campbell, P. G. C. and P. M. Stokes. 1985. Acidification and Toxicity of Metals to Aquatic Biota. Can. J. Fish. Aquat. Sci. 43:2034-2049.

Chester, R., W. M. Kudoja, A. Thomas, and J. Towner. 1985. Pollution reconnaissance in stream sediments using non-residual trace metals. Environ. Pollut. Ser. B. 10:213-238.

Duncan, D. A., and J. F. Klaverkamp. 1983. Tolerance and resistance to cadmium in white suckers (Catostomus commersoni) previously exposed to cadmium, mercury, zinc or selenium. Can. J. Fish. Aquat. Sci. 40:128-138.

Haines, T. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. Trans. Am. Fish. Soc.

110:669-707.

Herlihy, A. T., P. R. Kaufmann, M. E. Mitch, and D. D. Brown. 1990. Regional estimates of acid mine drainage impact on streams in the mid-Atlantic and southeastern United States. Water, Air, and Soil Pollution 50:91-107.

Kimball, K. D., and S. A. Levins. 1985. Limitations of laboratory bioassays: the need for ecosystem level testing. Bioscience 35:165-171.

Likens, G. E., R. F. Wright, J. N. Galloway, and T. J. Butler, 1979. Acid Rain. Scientific American 241:43-47.

Lowe, R. L. 1974. Environmental Requirements and Pollution Tolerance of Freshwater Diatoms. EPA-670/4-74-005. Office of Research and Development, USEPA, Cincinnati, OH.

Marouka, S., and S. Jamanaka. 1980. Production of mutagenic substances by chlorination of waters. Mutat. Res. 79:381-386.

Maurice, C. G., R. L. Lowe, T. M. Burton, and R. M. Stanford. 1987. Biomass and compositional changes in the periphytic community of an artificial stream in response to lowered pH. Water, Air, and Soil Pollution 33:165-177.

Mayer, F. L., and M. L. Ellersieck. 1986. Manual of Acute Toxicity: Interpretation and Database for 410 Chemicals and 66 Species of Freshwater Animals. U.S. Department of Interior, Fish and Wildlife Service/Resource Publication 160, Washington, D.C.

McCormick, P. V., and J. Cairns, Jr. 1992. Limitations to long-term species accrual development of microbial community structure in aquatic "islands." J. Freshwater Ecol. 7:381-397.

Melzian, B. D., and N. Jaworski. 1991. Toxicity of chlorine and ammonia to aquatic life: chemistry, water quality criteria, recent research, and recommended future research. In: Water Quality Standards for the 21st Century. USEPA, Office of Water (WH-553), Washington, D.C. pp. 127-138.

Nacci, D., E. Petrocelli, P. Commeleo, and W. Greene. 1990. Contribution of chlorination to the toxicity and persistence of chlorinated effluent from the Stamford (CT) sewage treatment plant. ERL-N Cont. No. 1118. USEPA, Environmental Research Laboratory, Narragansett, RI.

National Research Council. 1981. Testing for effects of chemicals on ecosystems. National Academy Press,

Washington, D.C.

Niederlehner, B. R. and J. Cairns, Jr. 1993. Effects of previous zinc exposure on pH tolerance of periphyton communities. Environmental Toxicology and Chemistry 12:743-753.

Patrick, R. 1978. Ecology of freshwater diatoms and diatom communities. In: The Biology of Diatoms (D. Werner, ed.). University of California Press, Berkeley, CA. pp. 284-332.

Peterson, H. G. and F. P. Healy. 1985. Comparative pH dependent metal inhibition of nutrient uptake by Scenedesmus quadricauda (Chlorophyceae). Journal of Phycology 21:217-222.

Petersen, R. C. 1992. The RCE: a riparian, channel, and environmental inventory for small streams in the agricultural landscape. Freshwater Biology 27:295-306.

Pratt, J. P., N. J. Bowers, B. R. Niederlehner, and J. Cairns, Jr. 1988. Effects of chlorine on microbial communities in naturally derived microcosms. Environ. Toxicol. Chem. 7:679-687.

Silvester, N. R., and M. A. Sleigh. 1985. The forces on microorganisms at surfaces in flowing water. Freshwater Biol. 15:433-448.

Stokes, P. M. 1986. Ecological effects of acidification on primary producers in aquatic systems. Water, Air, and Soil Pollution 30:421-438.

USEPA. 1985. Ambient water quality criteria for chlorine. EPA-440 5-84-030. National Technical Information Center, Springfield, VA.

USEPA. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. EPA-444 4-89-001. USEPA, Office of Water, Washington, D.C.

USEPA. 1990. Staff Report: Numeric Chlorine and Ammonia Standards. USEPA, Office of Water, Washington, D.C.

Ward, P. S. 1974. Carcinogens complicate chlorine question. J. Water Pollut. Control Fed. 46:2638-2640.

Webster, J. R., M. E. Gurtz, J. J. Hains, J. L. Meyer, W. T. Swank, J. B. Waide, and J. B. Wallace. 1983. Stability of stream ecosystems. In: Stream Ecology: Applications and Testing of General Ecological Theory (J. R. Barnes and G. W. Minshall, eds.). Plenum Press, New York. pp. 355-395.

List of Manuscripts, Presentations, and Supported Degrees

Manuscripts

Arnegard, M. E., P. V. McCormick, and J. Cairns, Jr. In prep. Resistance and resilience of stream periphyton assemblages to pH stress. To be submitted to Aquatic Toxicology.

McCormick, P. V., M. E. Arnegard, and J. Cairns, Jr. Submitted. A Field-Based Method for Quantifying the Response of Benthic Stream Communities to Chemical Stressors. Journal of Aquatic Ecosystem Health.

McCormick, P. V., M. E. Arnegard, and J. Cairns, Jr. In prep. Assessing regional variability in ecosystem sensitivity to anthropogenic stress: Stream periphyton responses to chlorine exposure. To be submitted to Ecological Applications.

McCormick, P. V., and J. Cairns, Jr. Submitted. An evaluation of the Use of Algal Indicators for Water Quality Assessments. Journal of Applied Phycology.

Presentations

McCormick, P. V., M. E. Arnegard, and J. Cairns, Jr. 1993. An in situ experimental method for assessing aquatic ecosystem responses to chemical stressors. Abstracts of the Third International Conference of Aquatic Ecosystem Health & the Ecological Significance of Bioassay Techniques, VPI&SU, Blacksburg, Virginia.

Arnegard, M. E., J. Cairns, Jr. E. E. Schiedt, and J. M. McMunigal. 1993. Comparison of the responses of periphyton communities to copper delivered either through the water column or via chemical-releasing substrates. Virginia Journal of Science. in press. Presented at the 71st Annual Meeting of the Virginia Academy of Science, Old Dominion University, Norfolk, Virginia.

Arnegard, M. E., P. V. McCormick, and J. Cairns, Jr. 1992. A new method for assessing the effects of toxicants on Aufwuchs communities in situ using chemical-diffusing substrates. NABS Bulletin, Vol. 9, No. 1. Presented at the 40th Annual North American Benthological Society Meeting, University of Louisville, Louisville, Kentucky.

Arnegard, M. E. and J. Cairns, Jr. 1992. A new method for assessing the effects of inorganic pollutants on algal communities in streams. Proceedings of the Third Annual Environment Virginia Symposium. pp. 42-46. Presented at the Third Annual Environment Virginia Symposium, VMI, Lexington, Virginia.

Arnegard, M. E., P. V. McCormick, and J. Cairns, Jr. 1991. The development of chemical-diffusing substrates for in situ periphyton community surveys. Virginia Journal of Science, Vol. 42, No. 2. Presented at the 69th Annual Meeting of the Virginia Academy of Science, VPI&SU, Blacksburg, Virginia.

Supported Degrees

Arnegard, M. E. 1993. Toxicant-Releasing Substrates: A New Method for Delivering Copper to Microbial Communities in situ. Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Master of Science in Biology. 131 pp.